Oxidation of Some Biologically Important Organic Substrates by Lipopathic Cr(VI) and Mn(VII): Kinetics and Mechanistic Study

A THESIS

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by

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CERTIFICATE

This is to certify that the thesis entitled "**Oxidation of some biologically important organic substrates by lipopathic Cr(VI) and Mn(VII): Kinetics and mechanistic study**" being submitted by **Ms. Sarita Garnayak**, to the National Institute of Technology, Rourkela, India, for the award of the degree of **Doctor of Philosophy** is a record of bonafide research carried out by her under my supervision and guidance. I am satisfied that the thesis has reached the standard fulfilling the requirements of the regulations relating to the nature of the degree. The contents of the thesis have not been submitted to any other university or institute for the award of any degree.

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Date:

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ABBREVIATIONS

BDMTDC	Benzyldimethyltelluronium dichromate
BTBAD	Bis-tetrabutylammonium dichromate
BTEAP	Benzyltriethylammonium permanganate
BTPPCC	Benzyltriphenyl phosphonium chlorochromate
BTPPD	Butyltriphenylphosponium dichromate
BuTPPCC	Butyltriphenyl phosphonium chlorochromate
CBZ	Carbamazepine
CBZ-DiOH	10,11-Dihydro-10,11-dihydroxy carbamazepine
CBZ-EP	Carbamazepine-10,11-epoxide
CCP	Chlorochromatoperiodate
CDCA	l-Chenodeoxycholic acid
CPCC	3-Carboxypyridinium chlorochromate
CTA	Cetyltrimethylammonium
CTAB	Cetyltrimethylammonium bromide
CTACN	Cetyltrimethylammonium cericnitrate
CTADC	Cetyltrimethylammonium dichromate
CTAP	Cetyltrimethylammonium permanganate
DCM	Dichloromethane
DCPCC	2,6- Dicarboxypyridinium chlorochromate
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	Diisobutylaluminum hydride
DMACC	Dimethylammonium chlorochromate
DMD	Dimethyl dioxirane
DMNB	Dimethylnitrobenzene
DMSO	Dimethylsulphoxide
DMT	Dimethoxytrityl
DSC	Differential scanning calorimetry
EBAFC	N-Ethylbenzylammonium fluorochromate

ECDCA	Ethylchenodeoxycholic acid
HBA	Hydrogen bond acceptor
HF	Hydrofluoric acid
INH	Isoniazid
KatG	catalase-peroxidase
KIE	Kinetic isotope effect
M. Pt.	Melting point
MBAFC	N-Methylbenzylammonium fluorochromate
MDR-TB	Multiple drug resistant tuberculosis
MSN	Mesoporous silica nanoparticles
Mtb	Mycobacterium tuberculosis
MTBAP	Methyltributylammonium permanganate
MTPPD	Triphenylmethylphosphonium dichromate
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NAPQI	*N*-acetyl-*p*-benzoquinone imine
NSAID	Nonsteroidal anti-inflammatory drug
OD	Optical density
ONSH	Oxidative nucleophilic substitution of hydrogen
PBC	Pyridinium bromochromate
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
PFC	Pyridinium fluorochromate
POMs	Polyoxometalates
PTC	Phase transfer catalysts
QFC	Quinolinium fluorochromate
SAR	Structure activity relationship
SDS	Sodium dodecylsulphate
TB	Tuberculosis
TBAB	Tetrabutylammonium bromide

TBABC	Tetrabutylammonium bromochromate
TBAC	Tetrabutylammonium chromate
TBACC	Tetrabutylammonium chlorochromate
TBACl	Tetrabutylammonium chloride
TBAD	Tetrabutylammonium dichromate
TBAFC	Tetrabutylammonium fluorochromate
TBAP	Tetrabutylammonium permanganate
TBPDC	Tetrabutylphosphonium dichromate
TEACC	Tetraethylammonium chlorochromate
TEMPO	2,2,6,6-Tetramethylpiperidinyloxy
TFA	Trifluoroacetic acid
TGA	Thermo gravimetric analysis
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMACC	Chlorochromate
TMAFC	Tetramethylammonium fluorochromate
TMEDADC	Tetramethylethylenediammonium dichromate
TPABC	Tetrapropylammonium bromochromate
TriBACC	Tributylammonium chlorochromate
TriPACC	Tripropylammonium chlorochromate
TriPAFC	Tripropylammonium fluorochromate
TX-100	TritonX-100
XDR-TB	Extensively-drug-resistant TB
XRD	X-ray diffraction

Preface

PREFACE

The search for novel oxidants has been ongoing due to the advancements in the synthesis of complex organic molecules under different reaction conditions. Most oxidation reactions employ inorganic oxidants with metal ions of Cr(VI), Mn(VII), Ce(IV), Fe(III), Ru(IV), V(V) etc. However, the poor solubility of these inorganic oxidants in nonaqueous media restricts their use for the oxidation of water insoluble organic substrates. To undertake reactions of organic substrates in organic homogeneous media, tailor made lipopathic oxidants are of much interest. Onium ions (generally refer to hypervalent ammonium, phosphonium, arsonium, tellurium ions etc,) having alkyl groups are attached as counterions to carry the oxidant from aqueous medium into organic medium. Due to amphipathic characteristics of these onium ions and resultant ion pair characteristics with the anionic counterpart the solubility, reactivity, and redox potentials of the oxidants vary significantly. These reagents are found to be mild, chemoselective and regioselective.

With an objective to develop new efficient, selective and mild oxidation protocols, the oxidizing ability of some lipopathic oxidants namely cetyltrimethylammonium dichromate (CTADC), cetyltrimethylammonium permanganate (CTAP) and cetyltrimethylammonium cericnitrate (CTACN) has been explored by our research group using cetyltrimethylammonium (CTA) ion as the onium counterion for anionic dichromate, permanganate and cericnitrate. The CTA ion is well known for its amphipathicity, which is having the characteristics of being solubilized in both aqueous and non-aqueous media. Unlike other quaternary ammonium ions (tetrabutyl or tetraoctyl ammonium ions), the CTA ion has a relatively small head group with more exposed charge and a well-balanced hydrophobic group to carry the ions to both water and organic media and thus is a magic amphiphile. Its balanced amphiphilic system makes it capable to form various artificial organized assemblies such as micelles, reverse micelles, microemulsions, vesicles etc. The preliminary reports on oxidation of various mono- and bi-functional

organic substrates by CTADC and CTAP show their mildness and chemoselectivity. The present research work aims at employing CTADC and CTAP to study the oxidative metabolism of some established drugs such as acetaminophen, epinephrine, isoniazid, carbamazepine and norfloxacin having multiple functional groups in nonaqueous medium.

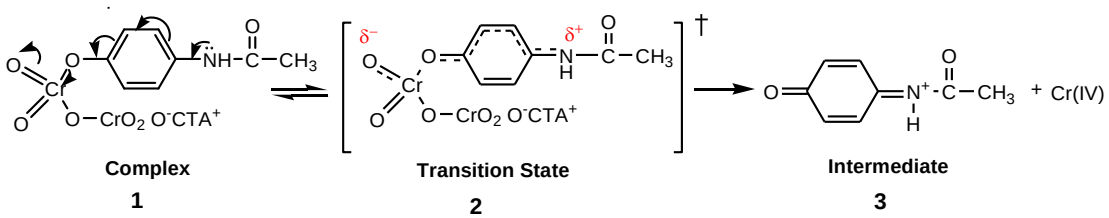
To achieve the objectives, the oxidation product(s) were isolated and characterized. The reaction kinetics was studied using UV-vis spectrophotometric method in different media with varied polarities and also in micro-heterogeneous systems generated due to the presence of surfactants. Suitable mechanisms have been proposed and supplemented by proper evidences such as deuterium kinetic isotope effect, solvent kinetic isotope effect, effect of medium, effect of temperature, effect of surfactants etc. The selectivity, mildness and biomimetic characteristics of the oxidative cleavage have been discussed and compared with that of the existing literature reports. Possibilities to utilize these oxidants as biomimetic chemical oxidation model have been explored primarily to synthesize the selective metabolites of drug candidates and to study the mechanism of oxidation at lipid-solution interfaces.

The present thesis entitled, "**Oxidation of some biologically important organic substrates by lipopathic Cr(VI) and Mn(VII) : Kinetics and mechanistic study**" comprises of seven chapters. Brief summary of the chapters are as follows.

A brief introduction on lipopathic oxidants has been presented in **Chapter 1**. Synthesis and applications of various lipopathic Cr(VI) and Mn(VII) oxidants have been discussed in this chapter with special reference to their mildness, selectivity and mechanism. Scope of the work along with the objective of the present thesis has also been presented in this chapter.

Chapter 2 deals with the oxidative cleavage of a well-known analgesic and antipyretic drug acetaminophen by a lipopathic oxidant, cetyltrimethylammonium dichromate (CTADC), in nonpolar medium and also in the presence of surfactants.

Oxidation of acetaminophen by CTADC in acetonitrile in the presence of acetic acid was found to produce *p*-benzoquinone and acetamide. Michaelis-Menten type kinetics was observed with respect to the substrate. An analysis of the reaction kinetics reveals the existence of a multistep complex mechanism, the first step of which involves a very fast formation of association complex (**1**) between acetaminophen and CTADC, followed by a rate-determining dissociation of the complex proceeding via a polar transition state (**2**) to form N-acetyl-p-benzoquinone iminium ion intermediate (**3**) (Scheme 1). Subsequent deprotonation and hydrolysis of the intermediate affords the final products. The proposed mechanism gets support from the effect of solvent polarity, isotope effect (solvent kinetic isotope effect and deuterium kinetic isotope effect) and the effect of temperature on the reaction rate.

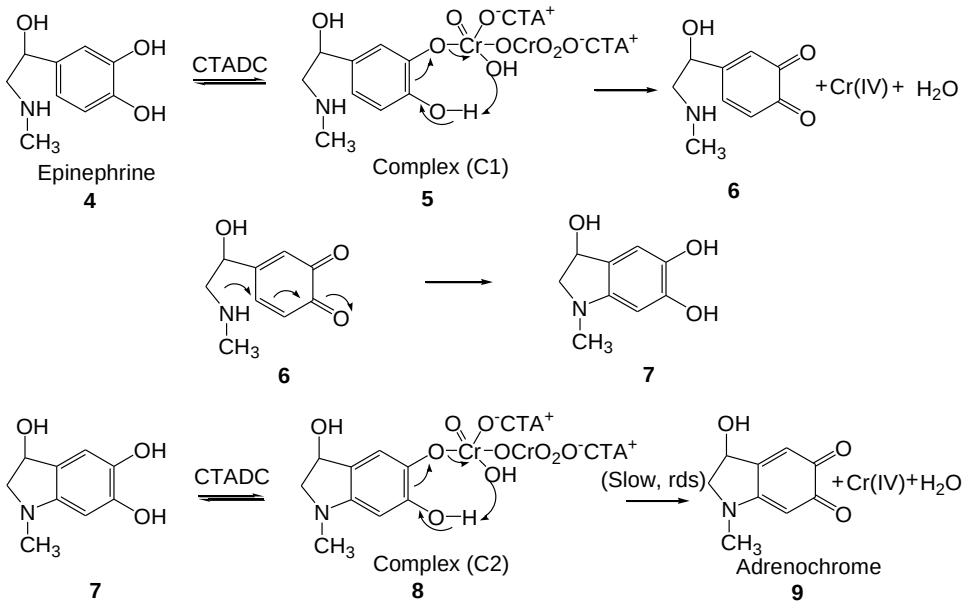


(Scheme 1)

Effect of various surfactants such as cetyltrimethylammonium bromide (CTAB), sodium dodecylsulphate (SDS) and triton-100 (TX-100) on the reaction rate proposes the formation of mixed reverse micelle type aggregates having different solubilization pockets for reactants. The substrate and the oxidant are thus partitioned into different types of environments, and accordingly the reaction is controlled.

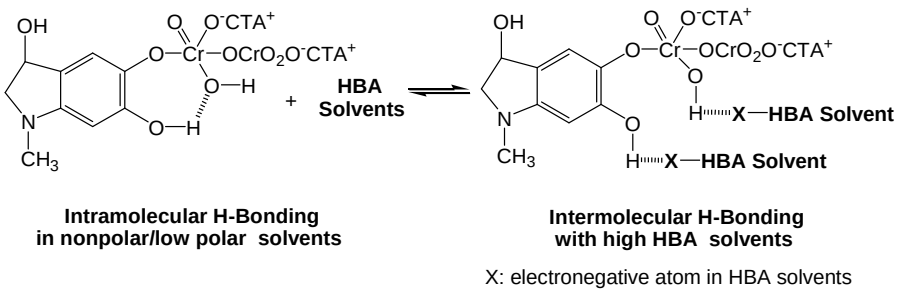
Chapter 3 of the thesis describes the oxidation of a neurotransmitter epinephrine (adrenaline), by CTADC. CTADC oxidizes epinephrine (**4**) to adrenochrome (**9**) selectively without affecting the secondary hydroxyl group present in the side chain of epinephrine. The rate of the reaction was measured by monitoring the product at 455 nm. A suitable ionic mechanism has been proposed (Scheme 2) basing on the experimental findings where epinephrine is first converted to epinephrine quinone (**6**). Intramolecular cyclization followed by aromatization through proton transfer affords leucochrome (**7**) which further oxidizes to the product

adrenochrome through the rate-determining decomposition of the complex C2 (**8**) via a less polar transition state.



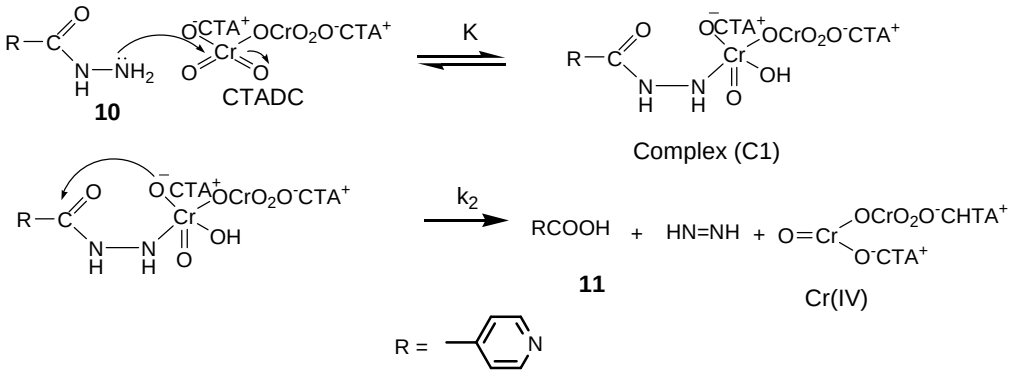
(Scheme 2)

The proposed mechanism gets support from the rate retarding effect in the presence of acetic acid and surfactant. The decrease in rate constant due to the increase in polarity and hydrogen bond acceptor (HBA) ability of the solvent indicates the existence of a less polar transition state and stabilization of the reactants through strong intermolecular hydrogen bonding (Scheme 3). Polar solvents with higher HBA ability stabilize the reactants and destabilize the transition state leading to an increase in energy of activation and lower rate of the reaction. Addition of surfactants (cationic, anionic and non-ionic) decreases the rate of reaction, which has been explained through the partition of oxidant and substrate in different microheterogeneous media.



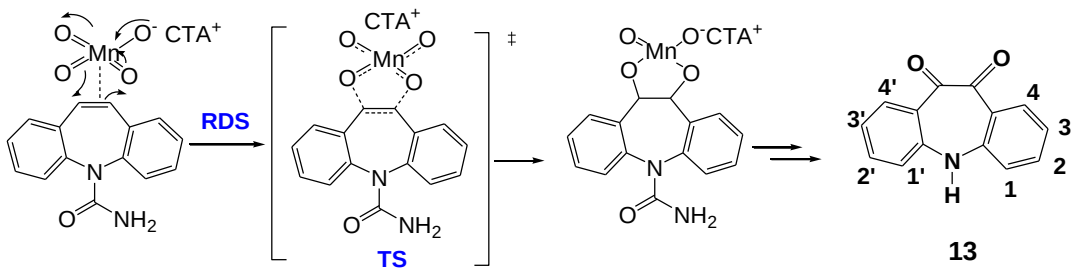
(Scheme 3)

The oxidation of anti-tubercular drug isoniazid (**10**) using CTADC has been presented in **chapter 4**. Isoniazid on oxidation in nonpolar medium generates isonicotinic acid (**11**) both in the presence and in the absence of acetic acid. Occurrence of Michaelis-Menten type kinetics with respect to isoniazid confirms the binding of oxidant and substrate to form a complex before the rate-determining step (Scheme 4). Existence of inverse solvent kinetic isotope effect ($k(H_2O)/k(D_2O) = 0.7$) in the acid catalyzed reaction provides evidence for a multistep reaction mechanism. Decrease in rate constant with the increase in [CTADC] reveals the formation of reverse micelle type aggregates of CTADC in non-polar solvents. In the presence of different ionic and non-ionic surfactants (CTAB, SDS and TX-100), CTADC forms mixed aggregates and controls the reaction due to the charge on the interface and partitioning of oxidant and substrate into two different domains. High negative entropy of activation ($\Delta S^{\ddagger}$ = -145 and -159 J K^{-1} mol^{-1} in the absence and in the presence of acetic acid) obtained by calculating the thermodynamic parameters proposes a more ordered and highly solvated transition state than the reactants. Furthermore, solvent polarity-reactivity relationship reveals (i) the presence of less polar and less ionic transition state compared to the reactants during the oxidation, (ii) differential contribution from nonpolar and dipolar aprotic solvents toward the reaction process and (iii) existence of polarity/hydrophobic switch at logP = 0.73 (log P refers to logarithm of partition coefficient between octanol and water). Nonpolar solvents contribute more to the reverse micellization whereas polar solvents contribute to the stability of reactants and transition state. A suitable mechanism has been proposed on the basis of experimental results. These results may provide insight into the mechanism of isoniazid oxidation in hydrophobic environment and may assist in understanding the drug resistance in different location.



(Scheme 4)

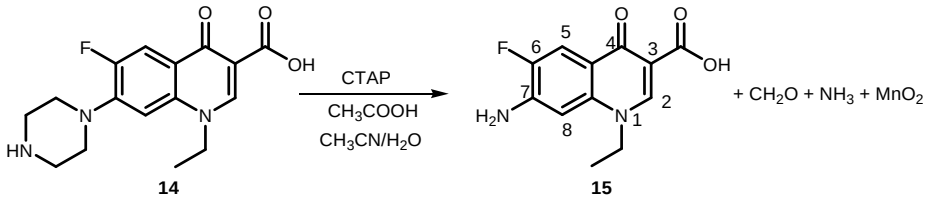
Chapter 5 deals with the oxidation of an established anticonvulsant and antiepileptic drug, carbamazepine (**12**) by cetyltrimethylammonium permanganate (CTAP) in non-polar medium. CTAP selectively converts carbamazepine to 1*H*-dibenzo[*b*,*f*]azepine-4,5-dione (**13**). The kinetics of the reaction was studied in organic media spectrophotometrically by monitoring the depletion of Mn(VII) at 530 nm. The reaction has been found to be fractional order with respect to carbamazepine and first order with respect to CTAP. Based on the experimental findings, a suitable mechanism has been proposed consisting of a rate determining *syn* addition of permanganate to the C=C double bond of carbamazepine to form Mn(V)-ester intermediate through a nonpolar cyclic transition state (Scheme 5). Subsequently, the intermediate decomposes and hydrolyses to the dicarbonyl product. The proposed reaction mechanism gets supportive evidences by the effect of solvent polarity and temperature on the rate of the reaction. Addition of ionic surfactants increases the rate of reaction. The catalyzing effect is explained through the possible formation of mixed reverse micellar aggregates where carbamazepine is partitioned more to the interfacial region in the vicinity of permanganate anion.



(Scheme 5)

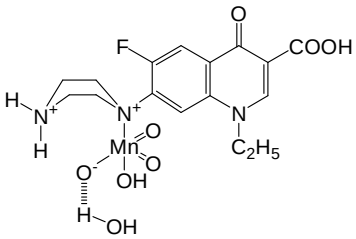
Chapter 6 describes the oxidative cleavage of an antibacterial drug norfloxacin (**14**) by CTAP in acetonitrile-water binary mixtures in the presence of acetic acid. The metabolized products have been identified as 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**15**), formaldehyde, and ammonia (Scheme 6). The kinetics of the reaction was studied by monitoring the absorbance of Mn(VII) at 530 nm under pseudo-first-order condition. The reaction has been found to be first-order with respect to CTAP and fractional order with respect to norfloxacin and acetic acid (Equation 1). Michaelis-Menten type kinetics with respect to norfloxacin confirms the binding of oxidant and substrate to form a complex before the rate determining step. Based on the experimental findings, a suitable ionic mechanism has been proposed. The proposed reaction mechanism has been supported by the effect of solvent polarity and effect of temperature on the reaction rate. High negative entropy of activation ($\Delta S^{\neq}$ = -259 to -158 J K^{-1} mol^{-1}) proposes the existence of a forced, more ordered and extensively solvated transition state. Further, the study on solvent polarity-reactivity relationship reveals (i) the presence of less polar transition state compared to the reactants, (ii) differential contribution from dipolar aprotic (acetonitrile) and polar protic (water) solvents toward the reaction process through specific and nonspecific solute-solvent interaction and (iii) the presence of intramolecular hydrogen bonding in oxidant-substrate complex in acetonitrile rich domain and intermolecular hydrogen bonding between oxidant-substrate complex and water in water rich domain (Scheme 7).

$$\text{Rate} = 0.15\,[\text{CTAP}]^{0.99}\,[\text{Norfloxacin}]^{0.73}[\text{Acetic acid}]^{0.59} \qquad (1)$$

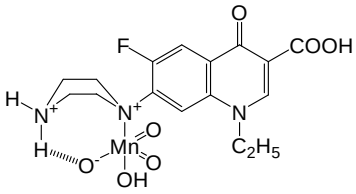


14 **15**

(Scheme 6)



Strong intermolecular hydrogen bonding in water rich domain



Strong intramolecular hydrogen bonding in acetonitrile rich domain

(Scheme 7)

Summary and the future scope of the present research work have been presented in **Chapter 7**. The work presented in the thesis has been proved to be a successful attempt on establishing CTADC and CTAP as mild, chemoselective, lipopathic and biomimetic oxidants. The uniqueness in terms of reactivity, selectivity and difference with that of other classical and non-classical oxidants has been attributed to the presence of CTA^+ which tunes the reactivity of the anionic oxidizing counterpart through tight ion pair formation in nonpolar medium. These oxidants may serve as a non-enzymatic, chemical oxidation model primarily to synthesize the selective metabolites of drug candidates in the course of drug discovery and development. Elucidation of probable mechanistic pathway of drug oxidation using these oxidants in the presence of artificial organised assemblies may serve as a reasonable model to enlighten the complex biological reactions occurring at the lipid–solution interface in the living system and give valuable information for finding more suitable drug candidate to fight with oxidative stress and to combat drug resistance.

Chapter 1

A Brief Review on Lipopathic Oxidants of Cr(VI) and Mn(VII)

1.1 INTRODUCTION

Oxidation is one of the fundamental reactions in synthetic organic chemistry having importance in both academia and industry, and there is always a demand for selective and mild oxidation methods. Indeed, numerous research groups have directed their efforts towards the development of novel oxidation processes and the trend is continuing. Most of the oxidants are based on oxo derivatives of inorganic transition metals like Cr(VI), Mn(VII), Ce(IV), Fe(III), Ru(IV), V(V) etc. However, the poor solubility of these inorganic oxidants in nonaqueous medium restricts their uses for the oxidation of water insoluble organic substrates and, thus it leads to the engineering of many novel oxidants among which the phase transferring oxidants or lipopathic oxidants are gaining wide interest. The discovery of phase transfer catalysts (PTC) such as quaternary ammonium salts which can transport the active oxidant species into organic phase helped to extend their use in organic synthesis to a great extent.[1-5] These active anionic oxidants are transferred to the organic phase in the form of soluble salt or an ion pair with PTC (Scheme 1.1). This possibility has attracted enormous interest from synthetic as well as mechanistic point of view. In some experiments, the soluble salts are formed in phase-transfer processes in biphasic medium and are utilized *in situ* without isolation, while sometimes, the salts are first isolated in aqueous medium and are then dissolved in the desired nonaqueous solvents.[3-5]

$$\underset{(aq)}{R_4N^+X^-} + \underset{(aq)}{KMnO_4} \rightleftharpoons \underset{(org)}{R_4N^+\,MnO_4^-} + \underset{(aq)}{KX}$$

(Scheme 1.1)

To convert the inorganic oxidants lipopathic, onium ions (generally refer to hypervalent ammonium, phosphonium, arsonium, tellurium, halonium ions etc,)[6] having alkyl groups are linked as counterions which help in carrying the oxidant from aqueous medium into organic medium.[7-9] Utilization of onium ions as phase transfer catalyst is widely explored in literature. Due to amphipathic characteristics of

these onium ions and resultant ion pair characteristics with the anionic oxidants in different solvents, the solubility, reactivity, and redox potentials of these oxidants vary significantly. [10-28] These reagents are found to be mild, chemoselective and regioselective.[7-28] In different reaction conditions, sometimes these oxidants containing long chain alkyl onium ions show biomimetic characteristics, due to the counter ions, providing a micro-heterogeneous environment with different solubilization pockets for the substrates as in case of micelles, reverse micelles, microemulsions, vesicles for artificial systems, and proteins and lipid membranes in living systems.[10-17]

With an objective to develop efficient oxidation protocol a great deal of efforts in research has been directed toward the development of new oxidants with these onium ions. In this regard, oxidizing system containing onium ions and transition metal oxo-species of Mn(VII) and Cr(VI) play a vital role since Mn(VII) and Cr(VI) are known for the versatility to carry out a wide spectrum of synthetically useful oxidative transformations.[29-32] Herein, we have presented some important synthetic applications of these Mn(VII) and Cr(VI) based lipopathic oxidants containing onium counterions giving special reference to their mildness, selectivity and mechanism.

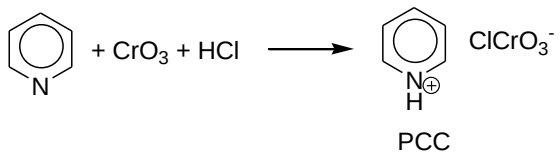
1.2 OXIDATION OF ORGANIC SUBSTRATES BY LIPOPATHIC Cr(VI)

Before the discovery of onium chromate or dichromate, water-soluble potassium or sodium dichromates were in use with strong acids as oxidants and in most cases the products were nonspecific. To make the reagents mild, the first attempt might be due to from Sarett school of research, who used pyridine to form salt with CrO_3, a Lewis acid, to oxidize some steroidal alcohols.[33] This reagent was subsequently used by various workers without analyzing the structure of the oxidant.[34-35] Corey, in his novel attempt for establishing pyridinium chlorochromate (PCC)[26] as a versatile oxidant, revisited to the Sarett's reagent and discovered it to be pyridinium dichromate (PDC).[27] Later on many heterocyclic ammonium ion based Cr(VI) oxidants were synthesized and their oxidation potential towards various

substrates were investigated. Synthesis and applications of various lipopathic Cr(VI) oxidants containing heterocyclic onium ions have been reviewed by Patel and Mishra in recent past.[7] Similarly a number of symmetric and asymmetric tetraalkylammonium ions with varying alkyl chain length have been synthesized in different research schools to serve as carriers of the oxidants and to deal with organic substrates in organic medium. Few applications of some of these lipopathic Cr(VI) oxidizing agents having heterocyclic and alkyl onium ions have been elaborated here to show their oxidizing ability, selectivity and mildness.

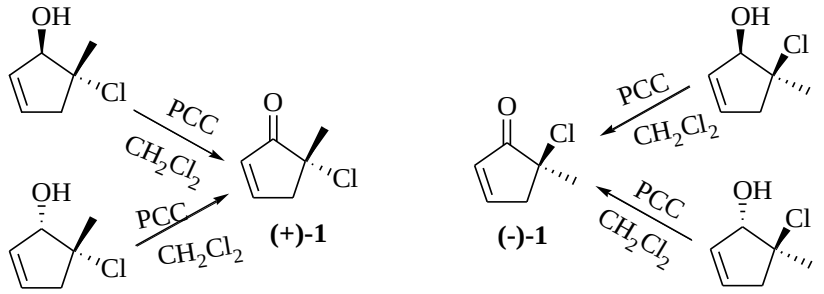
1.2.1 Heterocyclic ammonium halochromates and dichromates

Pyridinium chlorochromate (PCC) is one of the most important onium halochromates, well known for its versatility and selectivity in numerous oxidative transformations. It was first prepared by the addition of pyridine to an equimolar mixture of hydrochloric acid and chromium trioxide at 0^{o}C (Scheme 1.2).[26] It was extensively used for the selective oxidation of primary alcohol to aldehyde and secondary alcohol to ketone.

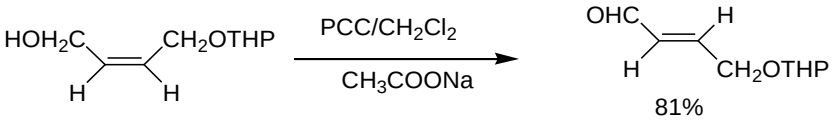


(Scheme 1.2)

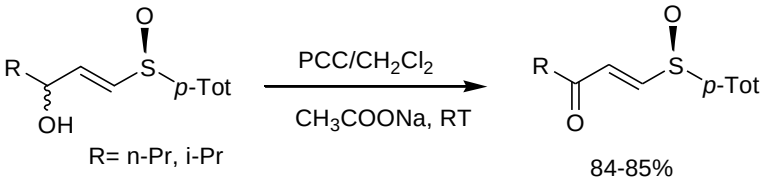
McMorris and Staake used PCC to oxidize the secondary hydroxy group of 5-chloro-5-methyl-2-cyclopenten-1-ol to corresponding ketone (**1**) without affecting the stereochemistry of the adjacent carbon (Scheme 1.3).[37] The presence of pyridinium ion makes PCC slightly acidic. The pH of 0.01M solution of PCC is 1.75.[38] Due to which the oxidations of compounds with acid sensitive group like tetrahydropyranyl ethers, the reaction mixture has to be buffered with powdered sodium acetate (Scheme 1.4).[26] Similar buffering methodology was used by Ordonez *et al*. for oxidizing (E)-γ-hydroxysulfoxide by PCC (Scheme 1.5).[39]



(Scheme 1.3)

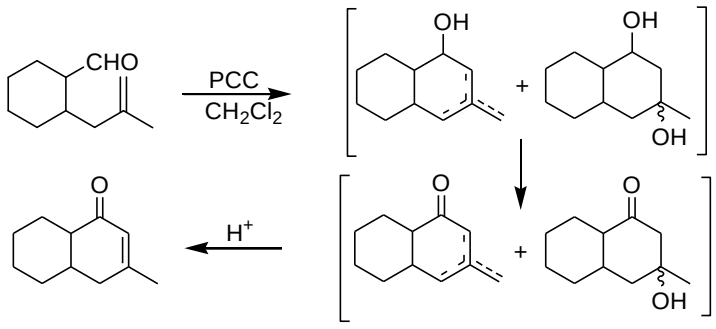


(Scheme 1.4)



(Scheme 1.5)

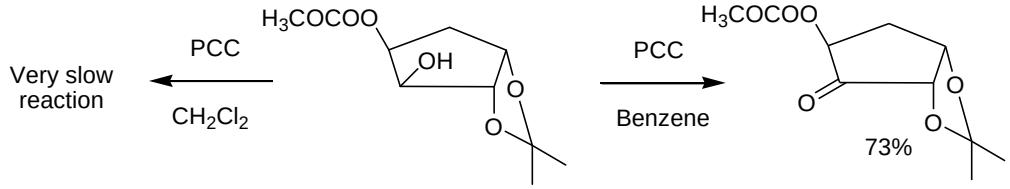
PCC was utilized by Corey and Boger for the oxidative cationic ring fusion of some cyclic unsaturated alcohols and aldehydes.[40] Efficient cyclization was observed only when the substrate is capable of affording a tertiary cation in the initial cyclic intermediate (Scheme 1.6).



(Scheme 1.6)

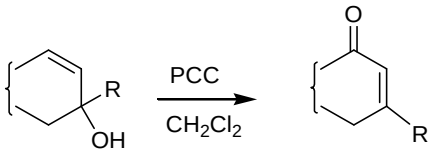
The reactivity of PCC was found to be solvent dependent. As shown in Scheme 1.7, the oxidation of secondary hydroxy groups to the corresponding carbonyl in sugars by using PCC was found to be inefficient in dichloromethane (DCM) while

in benzene the reactivity was increased to a large extent and corresponding cyclic ketones were obtained in good yield.[41]

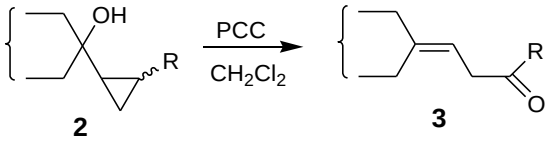


(Scheme 1.7)

Generally, tertiary alcohols are inaccessible for oxidation by oxidizing agents. However, with appropriate substituents PCC can transpose tertiary –OH group and subsequently oxidize to corresponding carbonyl compounds (Scheme 1.8).[42] The similarity in the reactivity of cyclopropyl ring system with the allyl moiety also led to a similar transpose mechanism during oxidation by PCC. Wada *et al.* used this protocol to convert the tertiary alcohol (**2**) with a cyclopropane ring to a β,γ-unsaturated ketone (**3**) (Scheme 1.9).[43]



(Scheme 1.8)



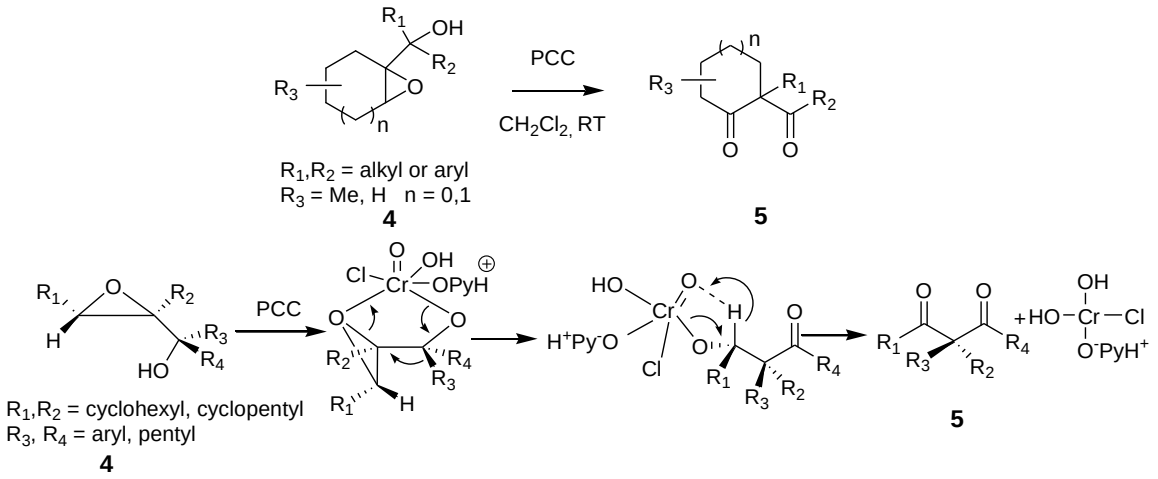
2 **3**

(Scheme 1.9)

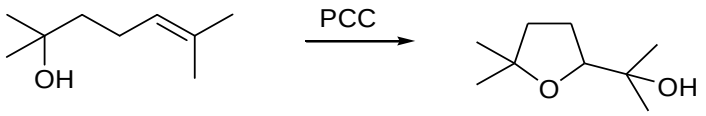
Ren *et al.* reported a facile one-pot synthesis of 1,3-diketone (**5**) with stereogenic quaternary center at C-2 position on the basis of the oxidative rearrangement of a series of α-hydroxy epoxides (**4**) in the presence of PCC and proposed a suitable mechanism for the conversion (Scheme 1.10).[44]

Beihoffer *et al.* used PCC for the intramolecular oxidative cyclization of bis homoallylic tertiary alcohols to yield substituted tetrahydrofuran products via the tethered chromate ester (Scheme 1.11).[45] The authors applied the method in several such tertiary alcohols which varied in the number and position of alkyl groups attached to the C=C. The relative reactivity of these substrates toward PCC was found to depend only on the number of R groups on the C=C, and not on the degree of

substitution on the most highly substituted alkene carbon. From this observation they suggested a symmetrical transition state in this intramolecular Cr(VI) alkene oxidation.

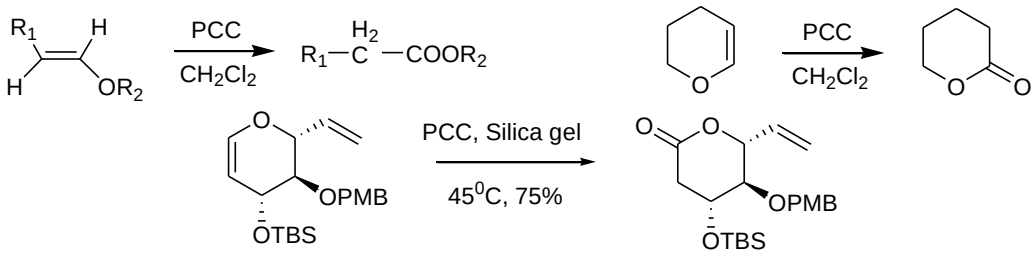


(Scheme 1.10)



(Scheme 1.11)

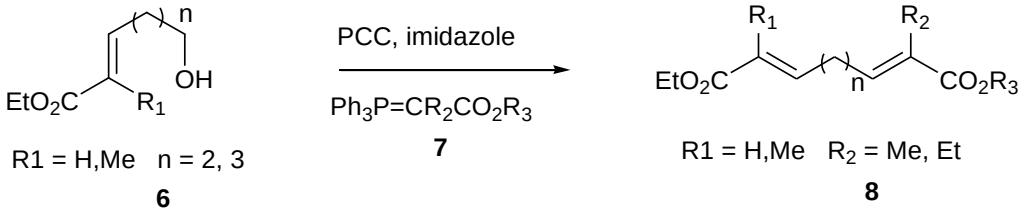
The Cr(VI) oxidation on the double bond is limited to alkenes or their derivatives. In contrast, PCC can be applied for the conversion of more electron rich enol ethers to esters and cyclic enol ethers to lactones (Scheme 1.12).[46]



(Scheme 1.12)

PCC in conjunction with silica gel was proved to be a better oxidant for organic substrates. In the synthesis of dienyl diesters (**8**) Phillips *et al.* used silica supported PCC for the oxidation of α,β-unsaturated hydroxy esters (**6**) followed by

trapping of the intermediate aldehydes with Wittig reagent **7** ($Ph_3P=CR_2CO_2R_3$) in a sequential one-pot procedure (Scheme 1.13).[47]



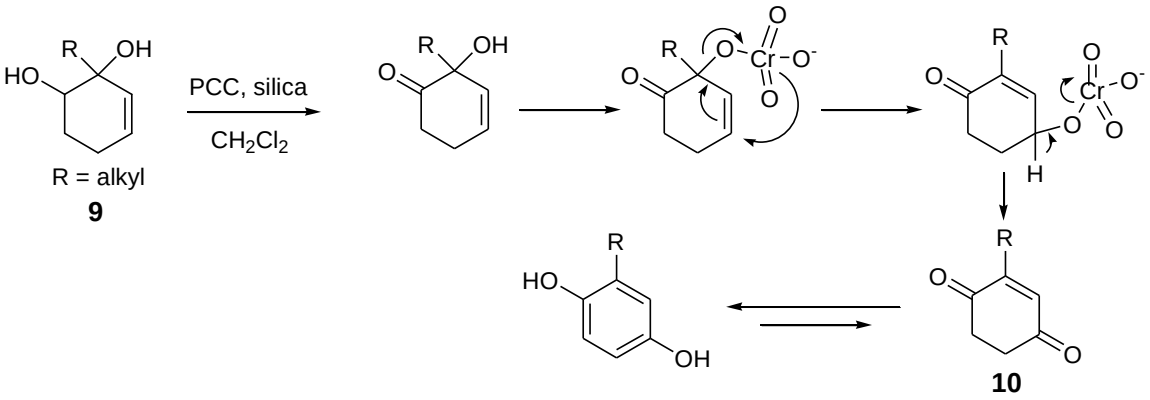
R1 = H,Me n = 2, 3
6

7

R1 = H,Me R_2 = Me, Et
8

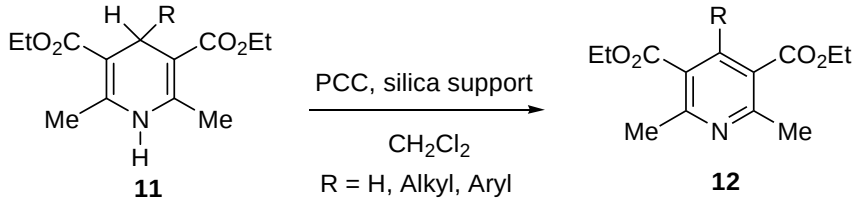
(Scheme 1.13)

PCC adsorbed on silica was applied to react with an allylic tertiary hydroxy group in 3-cyclohexene-1,2-diols (**9**) to yield 1,4-dihydrobenzoquinone (**10**).[48] Kim and Koo proposed a mechanism for the reaction wherein the secondary alcohol is initially oxidized to the corresponding ketone followed by subsequent transposition of tertiary allylic alcohol and its oxidation and enolization finally led to the product formation (Scheme 1.14).[48]



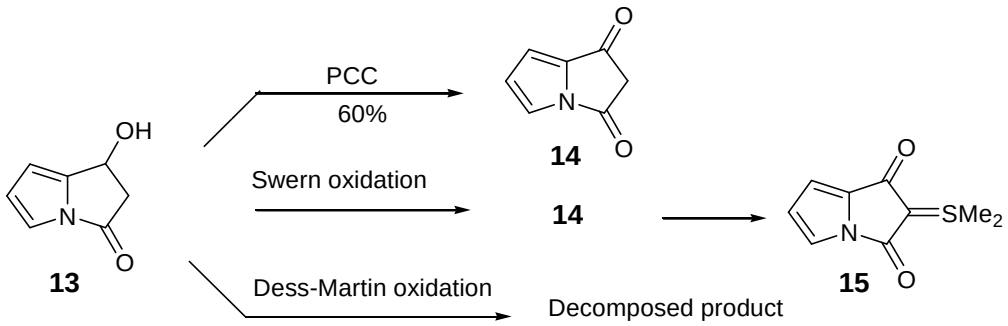
(Scheme 1.14)

By adsorbing PCC on various solid supports like alumina, silica gel and montmorillonite, Eynde *et al.*[49] investigated the aromatization of 1,4-dihydropyridines (**11**) to the corresponding pyridine (**12**) (Scheme 1.15) and found it to be more advantageous than aromatization in solution. The solid matrix helps in the isolation of pure product from the gummy mass, which is mostly obtained in solution.



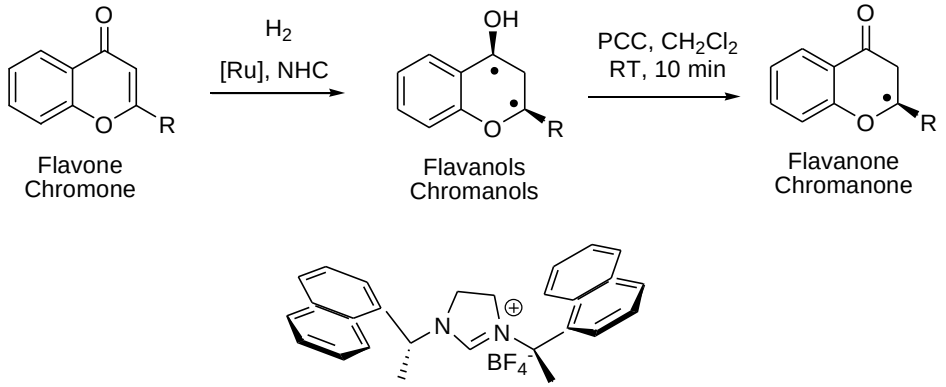
(Scheme 1.15)

McNab *et al.*[50] found PCC as an efficient oxidizing agent for the conversion of secondary alcohol **13** to the pyrrolizine-1,3-dione (**14**)**.** Efficient agitation of the reaction mixture throughout the oxidation along with magnetic stirring and bubbling nitrogen through the solution significantly increased the yield of the product. For the conversion of the **13** to **14** other oxidation methods met with little success. For example, Dess–Martin condition gave unidentified decomposition products. Swern oxidation converted the desired dione **14** to ylide **15** (Scheme 1.16)**.** Pyridinium dichromate (PDC) and manganese dioxide (MnO_2) mediated oxidation of **13** was successful but the yield was significantly lower.



(Scheme 1.16)

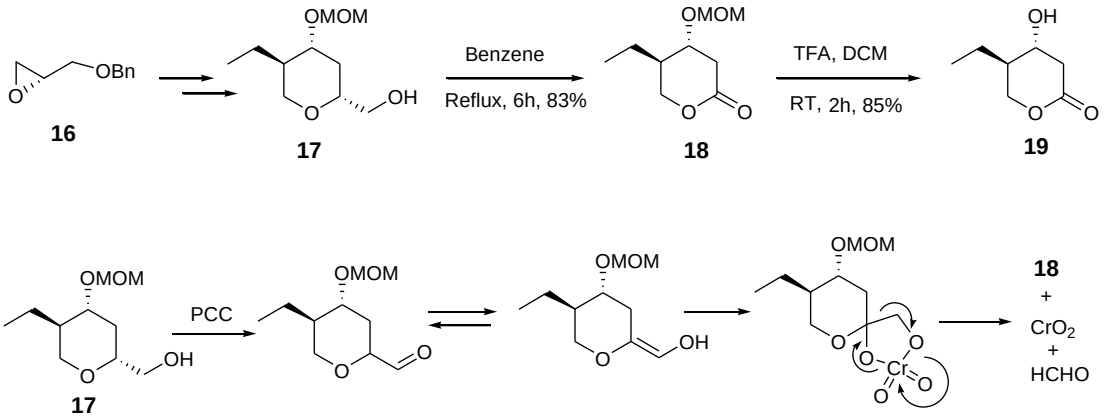
For the synthesis of various substituted enantiomerically enriched flavanones, and chromanones Zhao *et al.* utilized a two step strategy comprises of stereoselective hydrogenation followed by selective oxidation (Scheme 1.17).[51] To preserve the integrity of the newly formed stereocenter at C2, PCC was found to be the best choice as mild and selective oxidizing agent.



Chiral N-Heterocyclic cerbene (NHC) ligand

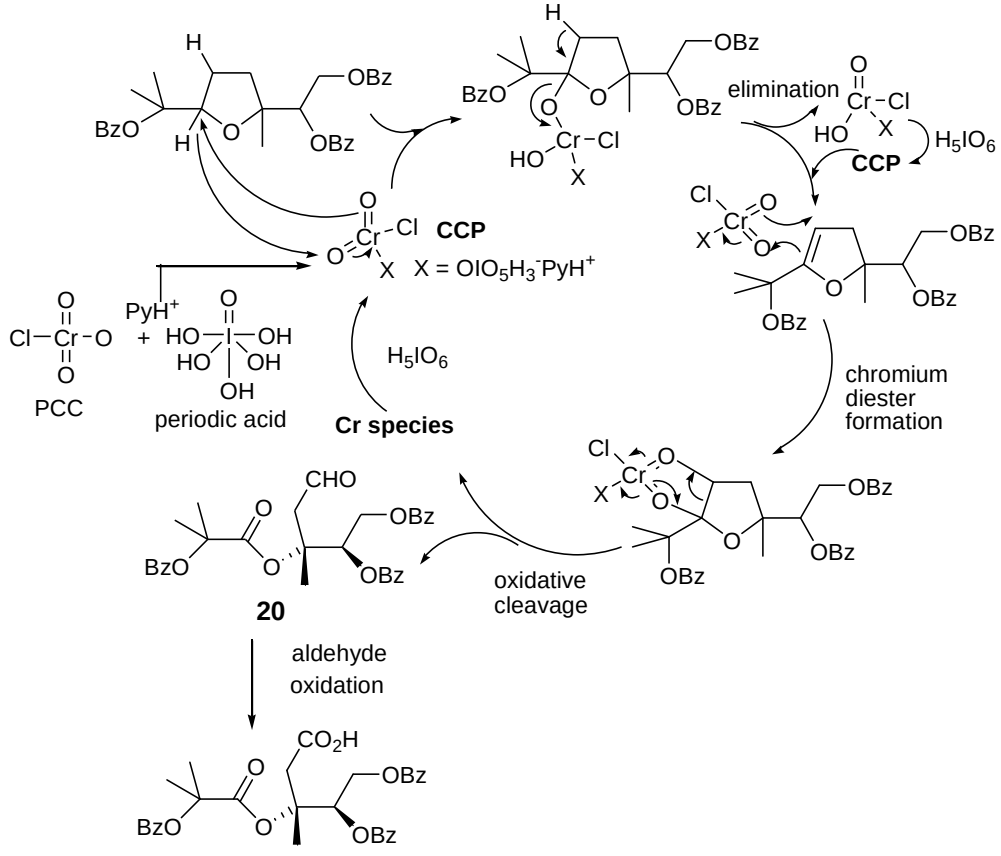
(Scheme 1.17)

Reddy *et al.*[52] reported a new synthetic route to δ-lactone, simplactone B (**19**) utilizing the highly stereoselective Prins cyclization and PCC mediated over oxidation from (R)-benzyl glycidyl ether **16** (Scheme 1.18). Oxidation of the cyclized alcohol **17** using excess of PCC in refluxing conditions in benzene resulted in the lactone **18** in 83% yield (as the only isolable isomer from column chromatography). Deprotection of MOM group of **18** using trifluoroacetic acid (TFA) in DCM furnished the target product simplactone B **19** in 85% yield. The oxidation has been presumed to proceed as shown in Scheme 1.18. Similar strategy for the synthesis of δ-lactone via PCC mediated oxidative cleavage of pyranyl methanols have been reported by Yadav *et al.*[53-54]

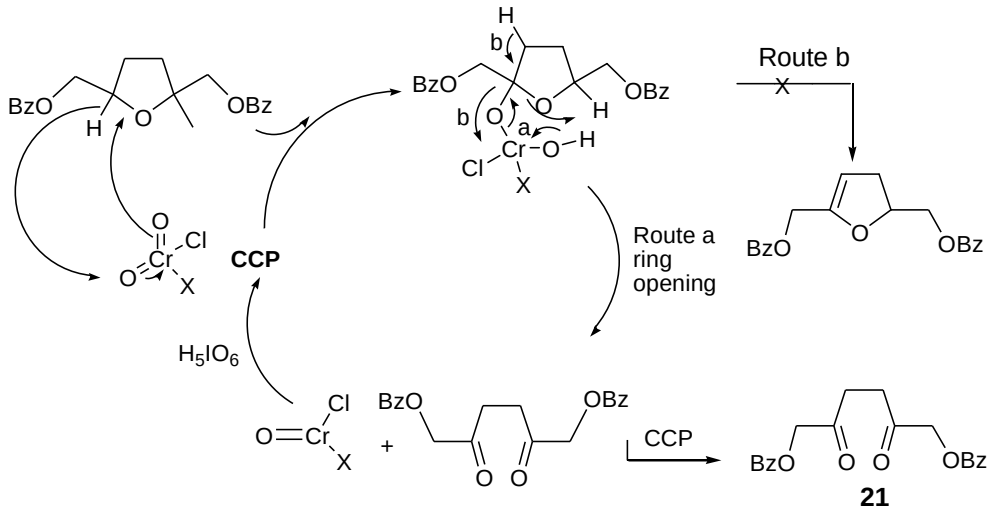


(Scheme 1.18)

As chromium(VI) species are known to be carcinogenic and environmentally hazardous, Piccialli *et al.*[55] have used a greener approach for the oxidation of some mono- and poly-THF compounds using the catalytic system PCC (cat.)/H_5IO_6. PCC with periodic acid (H_5IO_6) generates the active oxidant chlorochromatoperiodate (CCP). Novel oxidative pathways leading to the modification/degradation of tetrahydrofuran (THF) ring, as well as of the poly-THF backbone, have been described. Attack of the oxidant at the angular C–H bond of the target THF ring with formation of a mixed chromium ester has been proposed to be the first event followed by two main routes. Oxidative carbon-carbon bond cleavage, to give dicarbonyl products (**20**), was reported for 2,2,5-trisubstituted THFs while for 2,5-disubstituted mono-THFs, 1,4-Diketones (**21**) were found to be the only product. The proposed catalytic cycles for these two processes have been presented in Scheme 1.19 and 1.20.[55] Hunsen described similar greener oxidation of alcohols, glycosides and sulfides using PCC as the catalyst together with periodic acid as co-oxidant.[56]



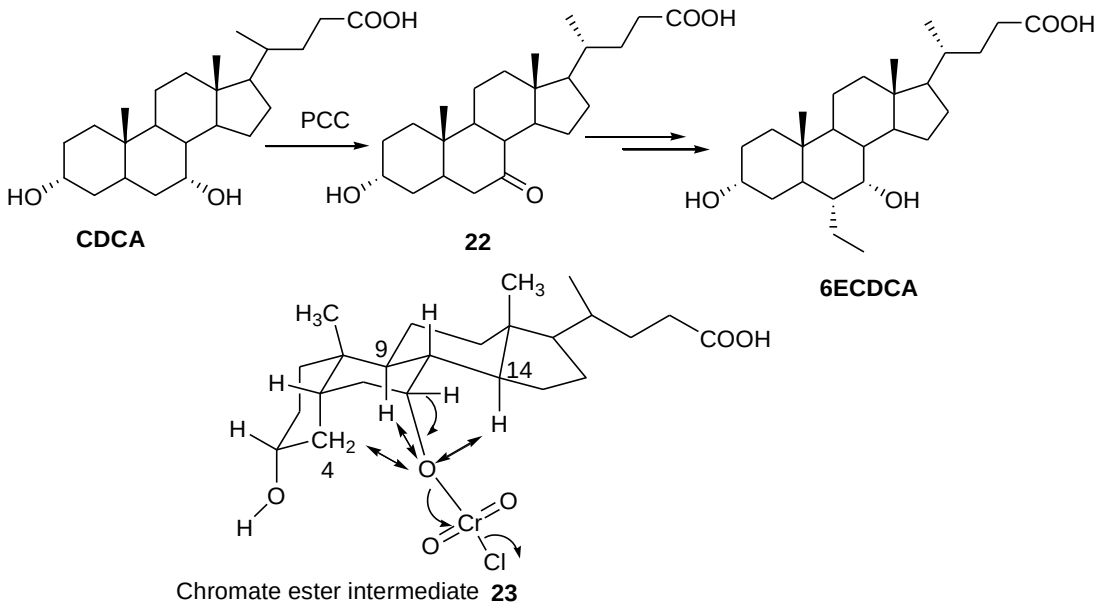
(Scheme 1.19)



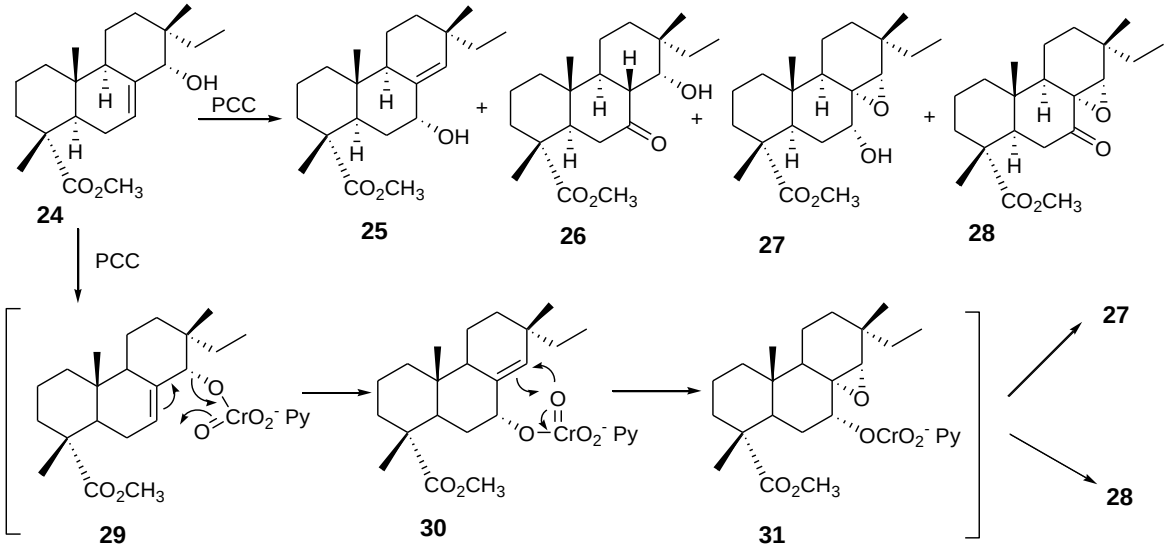
(Scheme 1.20)

For the synthesis of Farnesoid X Receptor (FXR) agonist 6a-ethylchenodeoxycholic acid (6ECDCA), PCC was used for the regioselective oxidation of one of the two hydroxyls of the l-chenodeoxycholic acid (CDCA). The regioselective oxidation of the axial 7-hydroxyl of CDCA with PCC in chloroform or DCM at room temperature afforded the desired 7-keto (**22**) in 82% yield (scheme 1.21).[57] The survival of the equatorial 3-hydroxyl can be explained through the higher ease of oxidation of axial hydroxyl groups by Cr(VI) oxidants than equatorial hydroxyls. During the removal of the chromate ester carbinol hydrogen, 1,3-diaxial strain (axial hydrogen from carbon 4, 9 and 14) gets relieved for an axial chromate ester (**23**), but not for an equatorial ester.

Oxidation of 14-α-hydroxydihydroisopimarate (**24**) by PCC was not particularly selective and formed a mixture of compounds, the ratio of which depended on the amount of oxidant used (Scheme 1.22).[58] Epoxy derivatives **27** and **28** could be formed as a result of isomerization of chromic ester **29** into 7-oxychromate **30** with subsequent formation of 8α, 14α-epoxychromate **31**, hydrolysis of which gave epoxy alcohol **27** and oxidation gave 7-keto-derivative **28**.



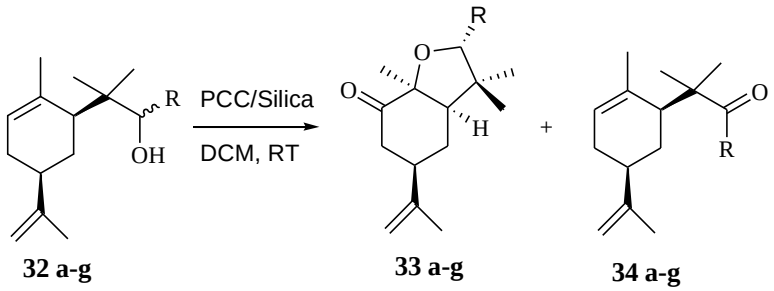
(Scheme 1.21)



(Scheme 1.22)

Oxidation of sterically crowded γ, δ-unsaturated primary and secondary alcohols (**32 a-g**) with 2.5 equivalents of PCC and silica gel in DCM at room temperature furnished the corresponding bicyclic ketones (**33 a-g**) in moderate to good yield as well as the expected aldehydes (**34 a-g**) (Scheme 1.23).[59] The bicyclic ketone (**33a**) was also found to form on oxidation of alcohol (**32a**) under various conditions, viz. PDC in DCM, PCC in the absence of silica gel and PCC and sodium

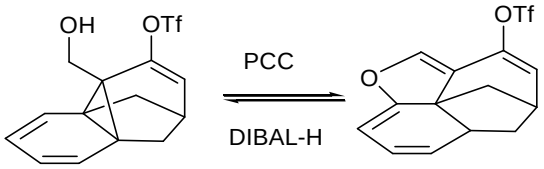
acetate. However, Jones reagent failed to generate the ketone **33a**, and furnished only a mixture of aldehyde **34a** and the corresponding acid which ruled out the role of the acidity of PCC in the formation of the bicyclic ketone. Similar strategy of the oxidation of γ, δ-unsaturated alcohols by PCC to afford bicyclic ketone was also applied by Heinrich *et al.* to carry out the oxidation of the etheno-bridged [4.3.1]propelladienol (**35**) to oxa[5.6.5.6]fenestratetraene (**36**). The reduction of **36** with diisobutylaluminum hydride (DIBAL-H) regenerated its precursor (Scheme 1.24).[60]



32 a-g **33 a-g** **34 a-g**

a: R= H, b: R= Me, c: R= Bu, d: R= Vinyl, e: R= Allyl, f: R= homoallyl, g: R= Benzyl

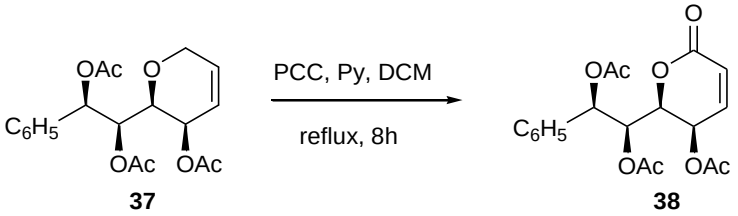
(Scheme 1.23)



35 **36**

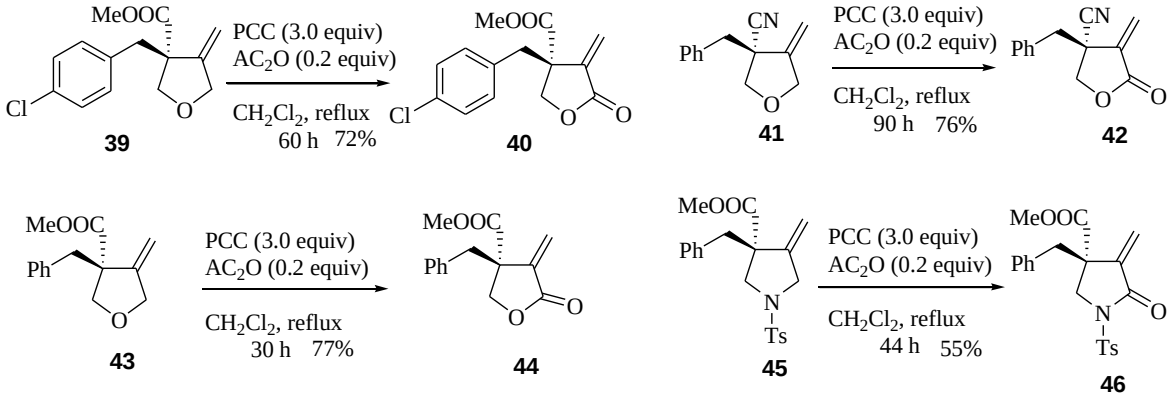
(Scheme 1.24)

For the selective conversion of densely functionalized ally ether (7R, 6S, 5S, 4R)-7-epi-Tri-acetoxy-3,6-dihydro-2H-pyran **(37)** to the corresponding (7R, 6S, 5S, 4R)-7-epi-triacetoxy(-)goniotriol **(38)** Srikanth *et al.* used PCC in DCM medium. PCC oxidized **37** to **38** in good yield without affecting the stereochemistry at neighboring carbons (Scheme 1.25).[61] Gowrishankar *et al.* have also reported similar chemo and regioselectivity in PCC mediated oxidation of the exo-methylene tetrahydrofuran derivatives (**39, 41, 43, 45**) to the corresponding β, β-disubstituted-α-methylene-γ-butyrolactones (**40, 42, 44, 46**) (Scheme 1.26).[62]



37 **38**

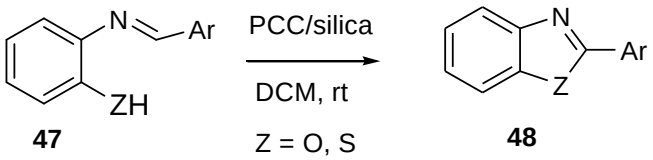
(Scheme 1.25)



39 **40** **41** **42** **43** **44** **45** **46**

(Scheme 1.26)

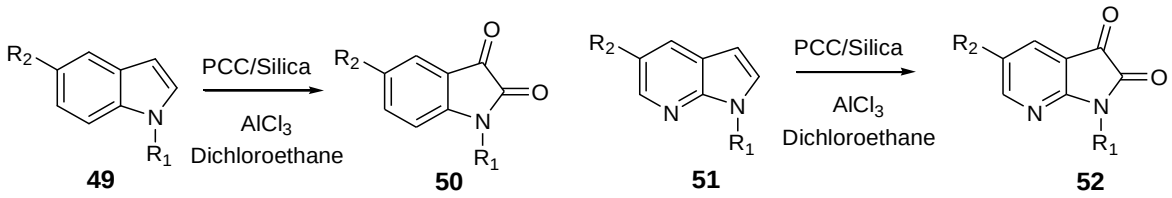
Silica gel supported PCC was successfully applied as heterogeneous oxidizing agent for the oxidative cyclization of structurally diverse thiophenolic and phenolic schiffs bases (**47**), thereby providing an efficient and convenient method for the synthesis of a library of 2-arylbenzothiazoles and 2-arylbenzoxazoles (**48**) in good to excellent yields (Scheme 1.27).[63]



47 **48**

(Scheme 1.27)

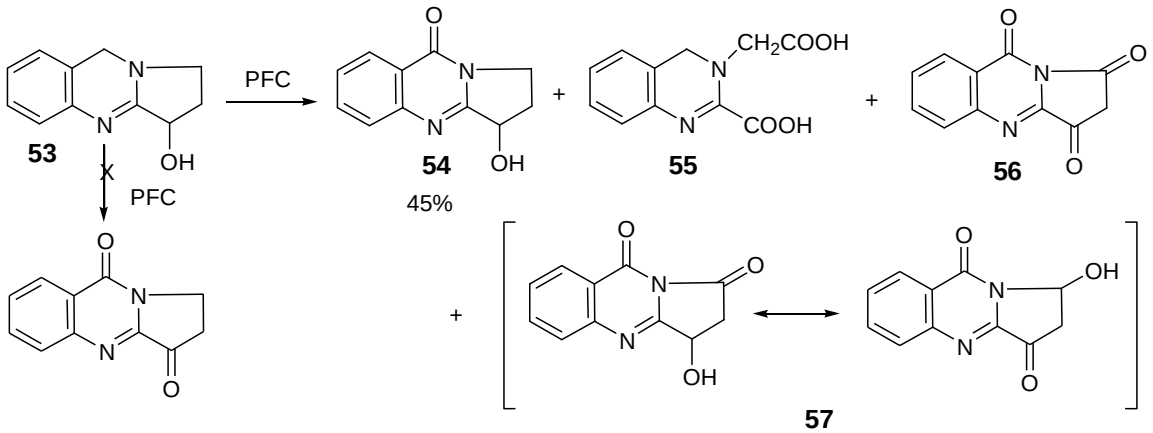
A simple and efficient method for the oxidation of indoles (**49**) and 7-azaindoles (**51**) to isatins (**50**) and 7-azaisatins (**52**) using PCC-silica gel with the aid of Lewis acid catalyst aluminium chloride ($AlCl_3$) in dichloroethane was described by Sriram *et al*. (Scheme 1.28).[64] Simplicity of the reaction conditions, easy workup procedure, and good yields are the key features of this protocol.



49 **50** **51** **52**

(Scheme 1.28)

Similar to PCC, Pyridinium fluorochromate (PFC) was prepared from a solution of CrO_3 in hydrofluoric acid (HF) and pyridine at an ice-cold temperature and was used for various oxidation processes.[38] It was used in the selective oxidation of secondary alcohols in the presence of primary alcohols and in the conversion of polycyclic hydrocarbons to cyclic ketones, benzoin to benzil, PPh_3 to $Ph_3P=O$, methylphenyl sulfide to corresponding sulfoxide, cyclohexanone oxime to cyclohexanone, deprotection of dioxolanes and dithiolanes to aldehydes and allylic Δ^5-steroid to the corresponding α,β- unsaturated ketone.[65] PFC was applied to a selective oxidation of secondary hydroxy groups in the presence of primary or secondary *tert*-butyldimethylsiloxy groups and the selectivity was found to be higher than that of the PCC.[66] The higher selectivity was attributed to the lower acidity of PFC than PCC.

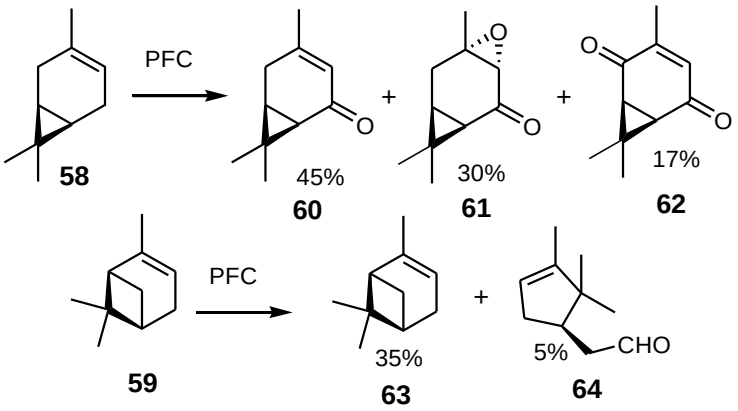


53 **54** **55** **56** **57**

(Scheme 1.29)

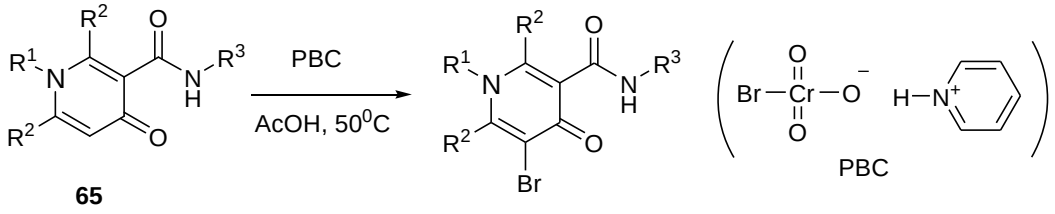
Versatility of PFC can easily be shown from the oxidation of vasicine (**53**). **53** was selectively converted to vasicinone (**54**) as the major product along with the minor products **55**, **56** and **57** (Scheme 1.29) when oxidized by PFC in acidic

medium.[67] Similarly oxidation of Δ^3-carnene (**58**) and α-pinene (**59**) with PFC afforded some novel oxidation products in acidic medium (Scheme 1.30).[68]



(Scheme 1.30)

Pyridinium bromochromate (PBC), another reagent of the onium halochromate family was used for the oxidation of several organic compounds such as benzhydrol, methionine, oxalic and formic acid, aliphatic aldehydes, benzyl alcohol, aliphatic alcohol, thioacids, organic sulfides, oximes and amino acids.[69] PBC can also be used for the bromination of aromatic compounds.[69-71] For the bromination of various poly-substituted 4-pyridones (**65**) to get the corresponding brominated product (**66**) via an oxidative nuclear bromination PBC was used as sole reagent (Scheme 1.31). In this case PBC performed the dual role of oxidant as well as source of bromonium ion.[71]

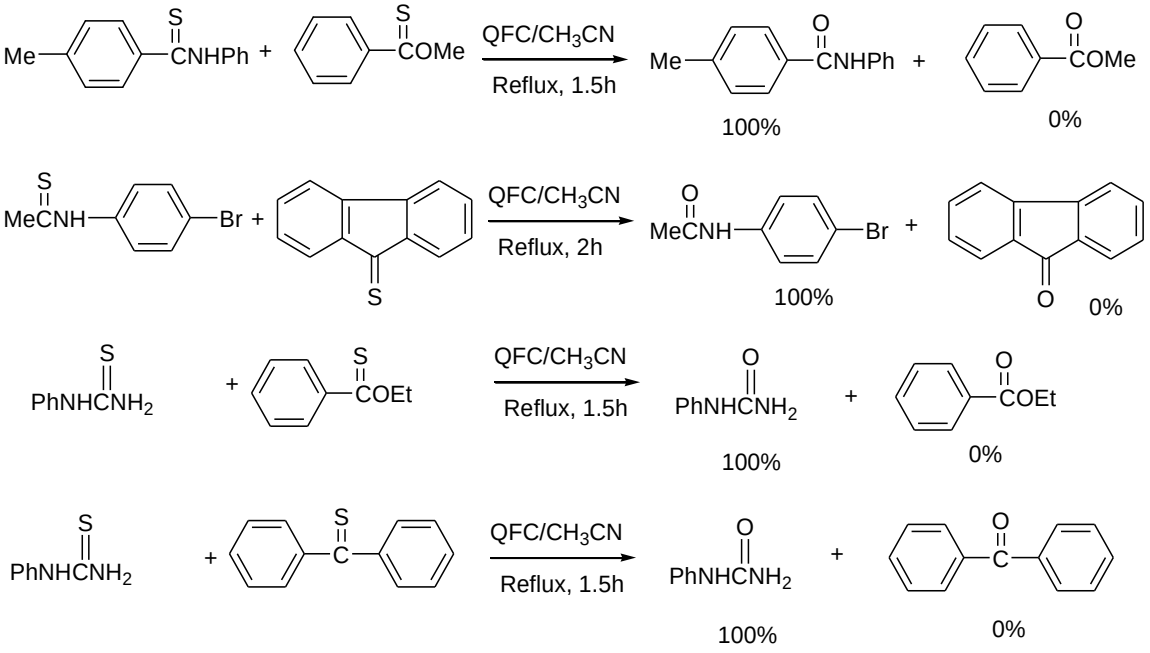


R^1 = aryl; R^2 = alkyl; R^3 = aryl or alkyl

(Scheme 1.31)

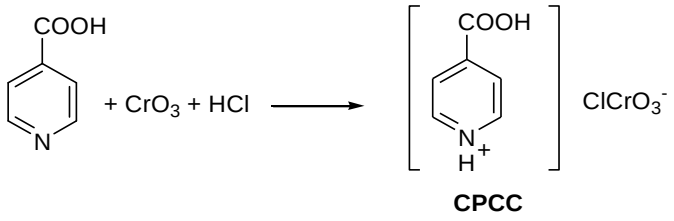
Quinolinium fluorochromate (QFC), can easily be prepared from quinoline, 40% aqueous HF and chromium trioxide in a molar ratio of 1:1.5:1.[72,73] QFC is acidic (pH of a 0.01 M solution: 2.65) but less pronounced than PCC (pH of a 0.01 M

solution: 1.75). Primary alcohols are found to be oxidized faster than secondary alcohol by this reagent.[74] In a competitive deprotection reaction of thioamides, thioureas, thionoesters and thioketones by QFC; thioamides and thioureas were selectively converted to the corresponding carbonyl groups in presence of the other two functional groups (Scheme 1.32).[75]



(Scheme 1.32)

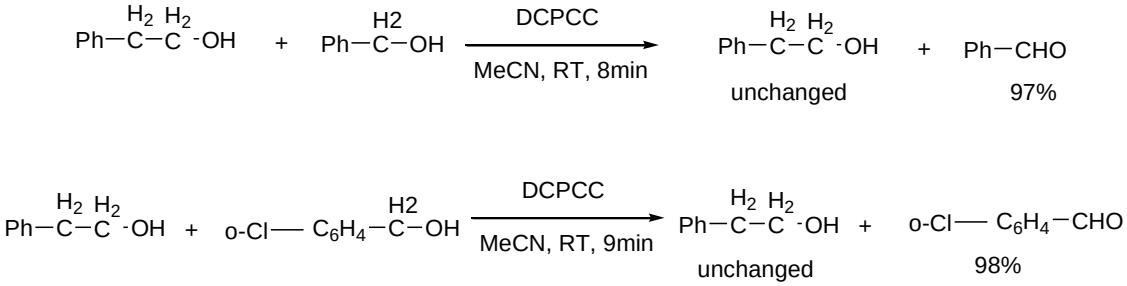
3-Carboxypyridinium chlorochromate (CPCC) prepared as per Scheme 1.33[76] was used for the efficient conversion of primary and secondary trimethylsilyl and tetrahydropyranyl (THP) ethers to their carbonyl compounds under nonaqueous condition. Trimethylsilyl ethers were oxidized selectively in the presence of tetrahydropyranyl ethers.[77]



(Scheme 1.33)

CPCC in the presence of aluminium chloride can selectively oxidize sulfides to sulfoxide and sulfones without effecting different functional groups including carbon-carbon double bonds, ketones, oximes, aldehydes, ethers, and acetals.[78] Aromatization of a variety of 1,4-dihydropyridines was conducted to get their corresponding pyridines in excellent yields by CPCC.[79] Different types of thioamides, thioureas, thiono esters and thioketones were deprotected to their corresponding carbonyl compounds with this reagent in good to excellent yields.

2,6- Dicarboxypyridinium chlorochromate (DCPCC) prepared by the reaction of pyridine-2,6-dicarboxylic acid with chromium trioxide in 6N hydrochloric acid was found to be less acidic than that of PCC.[80-82] This reagent was able to selectively oxidize the hydroxy group in presence of other oxidizable functional groups and benzylic alcohols in presence of other primary or secondary hydroxy groups (Scheme 1.34).[81] Selective deprotection of acetals or 1,1-diacetates in the presence of thioacetals at room temperature was also undertaken with this reagent.

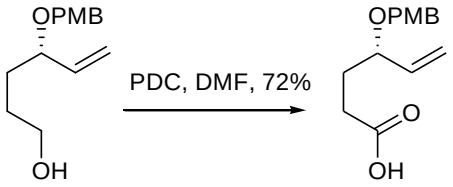


(Scheme 1.34)

Another noteworthy advantage of the reagent is the exclusive oxidation of oximes irrespective of the presence of semicarbazones or phenyl hydrazones. Oxidation of semicarbazones or phenyl hydrazones require a higher molar ratio of oxidant, much longer reaction time, and reflux temperature in acetonitrile and give low yield of products.[81] Heravi *et al.* found DCPCC as a highly effective oxidizing agent for the very fast conversion of dihydropyridines to pyridines under simple and relatively mild conditions in excellent yields.[83]

Pyridinium dichromate (PDC), another well-known and effective lipopathic Cr(VI) reagents was synthesized by gradual addition of pyridine to a cooled solution of chromium trioxide in water maintaining the temperature under 30^0C.[27] After dilution with acetone and cooling to - 20^0C, PDC was collected as orange crystals with a good yield. The high solubility in H_2O, DMF, DMSO, and DMA contributes to the versatility of the reagents for organic substrates. The reagent has been mostly used in DMF and DCM.

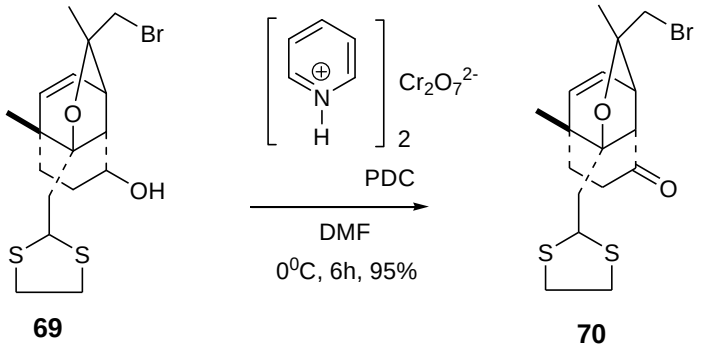
For oxidation of alcohols by PDC in DMF generally 1.25 equivalent of the oxidant is required to get corresponding carbonyl compounds with excellent yield without any side product, over oxidation or E to Z isomerization.[84-87] However with 3.5 equiv. of PDC in DMF, saturated primary alcohols are readily converted to carboxylic acids in good yields at room temperature through the intermediacy of isolable aldehydes.[88,89] This direct conversion of primary alcohols to carboxylic acids by PDC is convenient and is also possible in alcohols containing acid and base sensitive functionality.[90-92] For example, oxidation of the primary alcohol functionality of **67** with PDC in DMF afforded the corresponding acid (**68**) in 72% yield without effecting the protected hydroxyl group (Scheme 1.35).[93]



67 **68**

(Scheme 1.35)

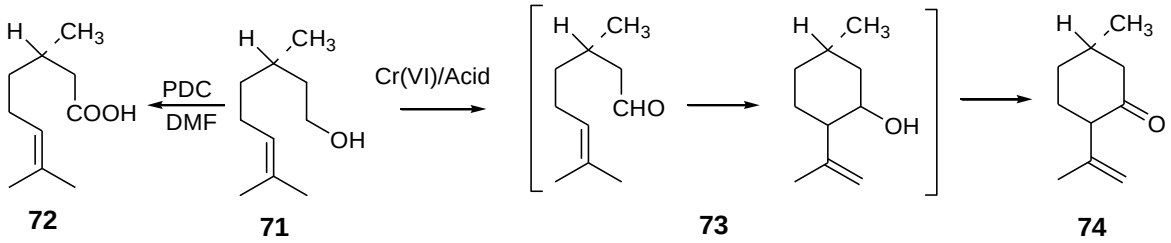
The mildness and selectivity of PDC is well documented from the oxidation of secondary hydroxyl group of **69** to yield the corresponding cyclic carbonyl compound **70** in 95% yield at 0 ^{0}C in DMF (Scheme 1.36). The sensitive thioacetal group was unaffected by this reagent. For the oxidation of **69,** PCC and Jones reagent were found to be nonspecific and the thioacetal unit was also affected by these reagents.[27]



69 **70**

(Scheme 1.36)

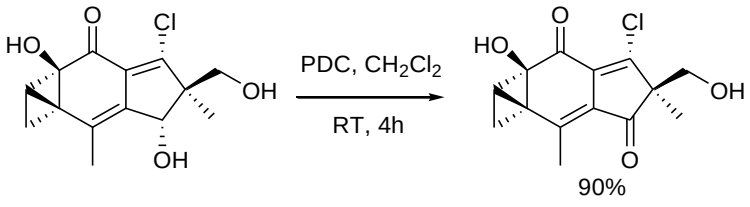
The oxidation of citronellol (**71**) to the corresponding acid (**72**) is another example of the mildness of the reagent. Under acidic conditions, the intermediate aldehyde (**73**) of citronellol undergoes cationic cyclization to form pulegone (**74**) (Scheme 1.37).[40]



72 **71** **73** **74**

(Scheme 1.37)

Arnone *et al.* reported selective oxidation of secondary alcohol over primary alcohol by PDC.[94] At room temperature when treated with PDC for 4 hrs **75** yielded **76** (Scheme 1.38).

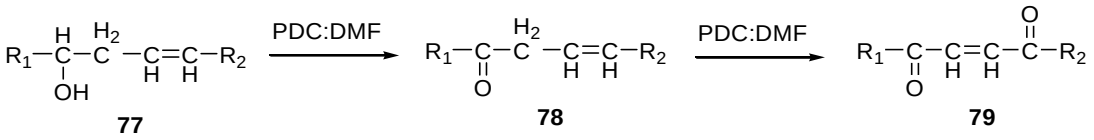


75 **76**

(Scheme 1.38)

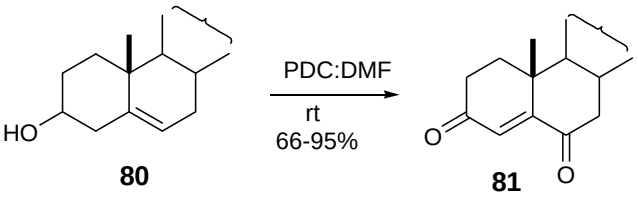
PDC in DMF oxidized derivative of homoallylic alcohol **(77)** to trans-enediones (**79**) in good yields (Scheme 1.39) and proposed to be proceed through the intermediacy of the ketone (**78**).[95] Hector *et al.* [96] also used PDC for the conversion of

various substituted steroidal homoallylic alcohol Δ^5-3β-alcohols (**80**) to the corresponding Δ^4-3,6-diketones (**81**) in good yields (Scheme 1.40).



77 **78** **79**

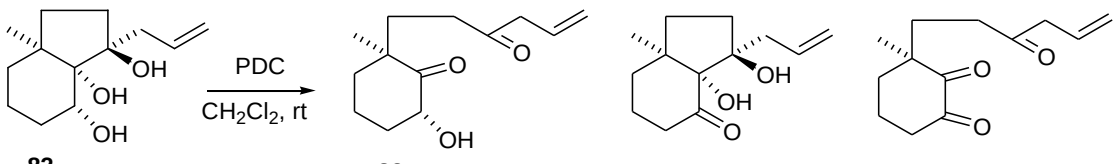
(Scheme 1.39)



80 **81**

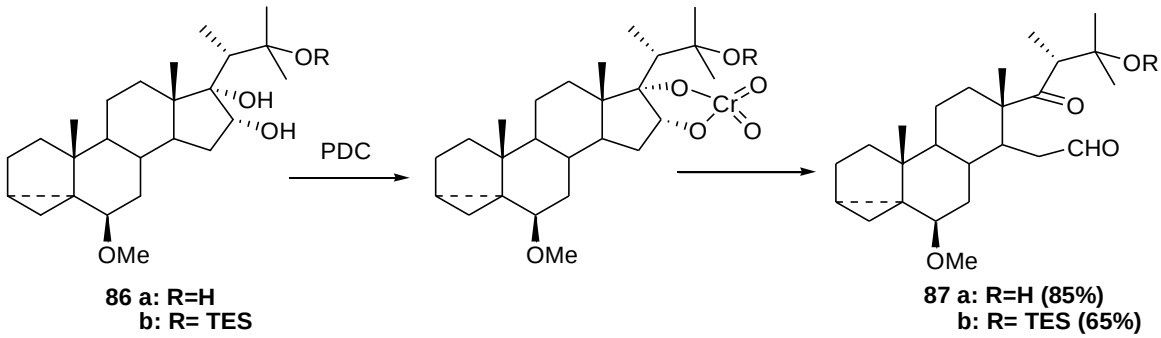
(Scheme 1.40)

Oxidation of triol (**82**) with PDC gave diketone (**83**) as the sole product in 93% yield instead of the expected ketone (**84**). Further, the stereochemistry of the secondary –OH group was not affected during the transformation. Even though the reaction time was increased to 19h using 10 equivalent of PDC, the corresponding triketone (**85**) was not formed (Scheme 1.41).[97] In the oxidation of triol (**86a**) by PDC with an aim to get the corresponding 16-oxo compound Morzycki *et al.* rather isolated D-seco-aldehyde (**87a)** in 85% yield even in presence of excess of the reagent. The fragmentation of a cyclic chromate ester intermediate was proposed for the formation of this product (Scheme 1.42).[98]



82 **83** **84** **85**

(Scheme 1.41)

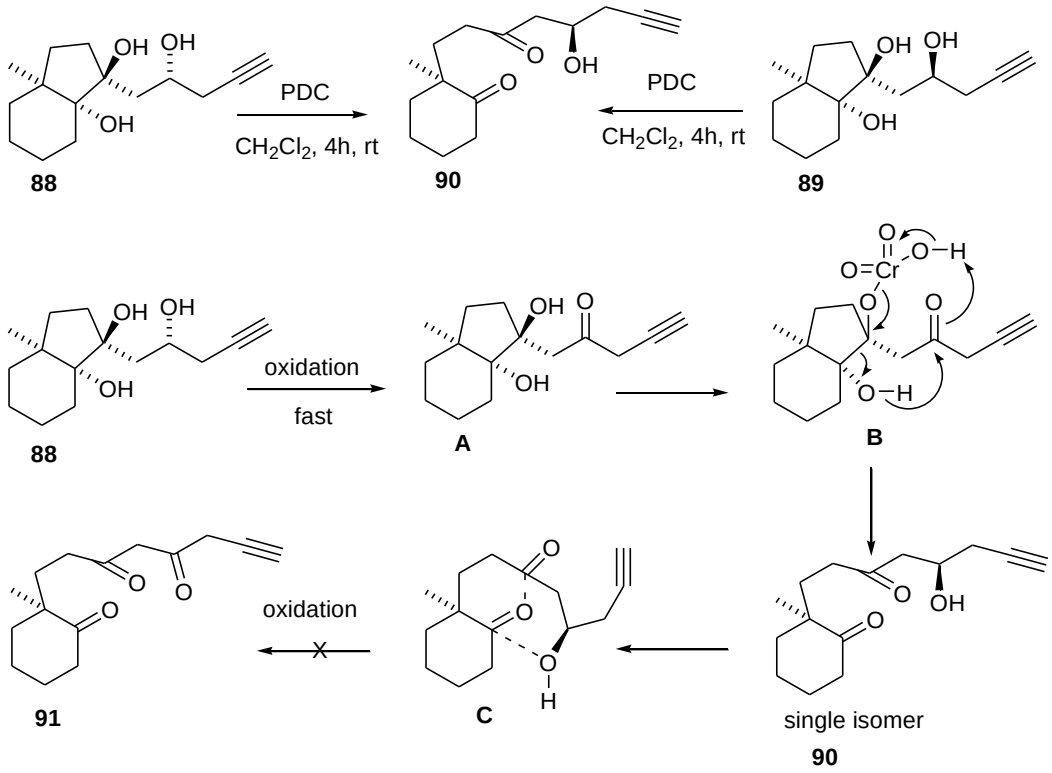


86 a: R=H
b: R= TES

87 a: R=H (85%)
b: R= TES (65%)

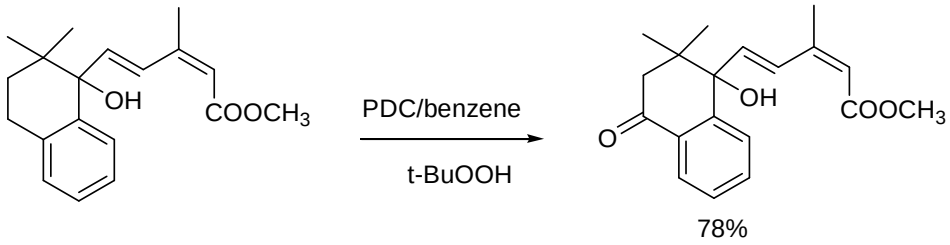
(Scheme 1.42)

When the diastereomeric triols **88** and **89** were subjected to oxidation with PDC, interestingly a single product **90** was formed (Scheme 1.43).[97] A probable reaction mechanism was proposed in which the selective oxidative cleavage of vicinal tertiary diols is accompanied by remote asymmetric induction and the stereochemistry of the secondary hydroxyl group is controlled. Again, the triketone (**91**) might not be produced due to the stabilization of the sterically hindered hydroxyl group as shown in structure 'C'.



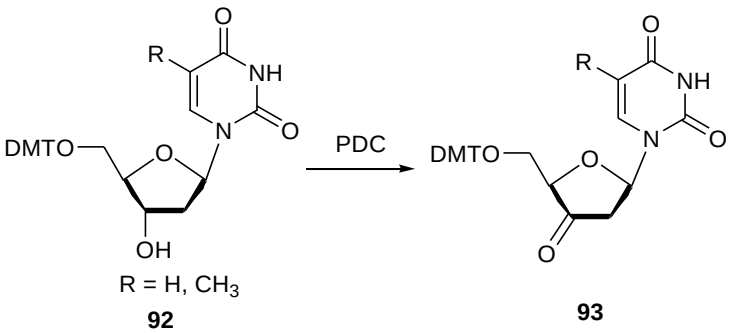
(Scheme 1.43)

Nyangulu *et al.* used PDC in benzene in presence of *tert*-butylhydroperoxide and celite to oxidize various alkyl-substituted aromatics at benzylic carbon hydrogen bond to furnish a ketone (Scheme 1.44).[99]



(Scheme 1.44)

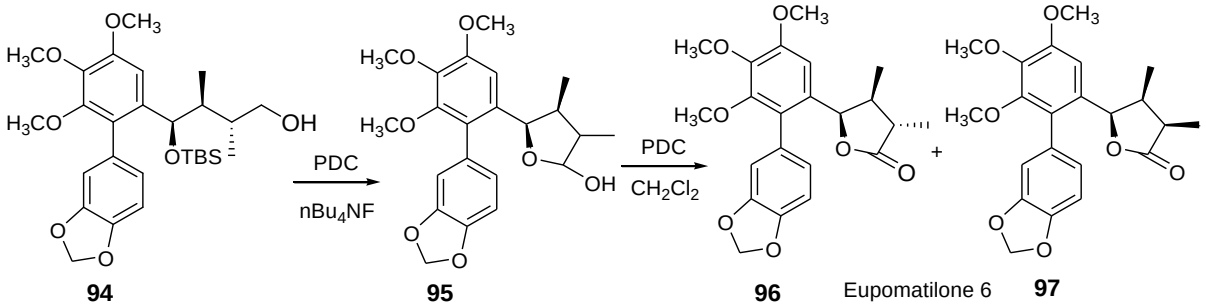
PDC in DCM selectively oxidized primary trimethylsilyl ether in presence of secondary trimethylsilyl ether.[100] *tert*-Butyldimethyl and *tert*-butoxydiphenylsiloxy groups are found to be stable under these conditions. Thus, an alcohol can be selectively oxidized to the carbonyl compound in presence of *tert*-butyldimethylsiloxy groups. Similar selectivity has been observed in presence of triisopropyl-, tert-butyldiphenylsiloxy, and tributylsiloxy groups. Selective oxidation of the –OH group by PDC in presence of dimethoxytrityl (DMT) ether was undertaken to oxidize 2'-hydroxy of thymidine (**92**) to the corresponding ketone (**93**) (Scheme 1.45).[101]



(Scheme 1.45)

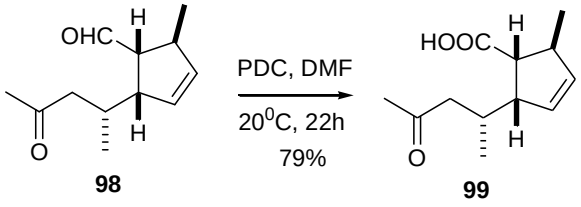
Coleman *et al.* for the synthesis of eupomatilone 6 (**96** and **97**) used PDC for the selective oxidation of **94** to corresponding aldehyde (85% yield). The aldehyde formed in the first step cyclized to lactol, **95** after fluoride-mediated deprotection of the silyl ether in THF. Compound **95** was produced as a mixture of diastereomers, presumably from epimerization of the intermediate aldehyde under basic reaction

conditions. Lactol to lactone oxidation in DCM afforded a 1:3 mixture of **96** and its epimer, **97** (Scheme 1.46).[102]



(Scheme 1.46)

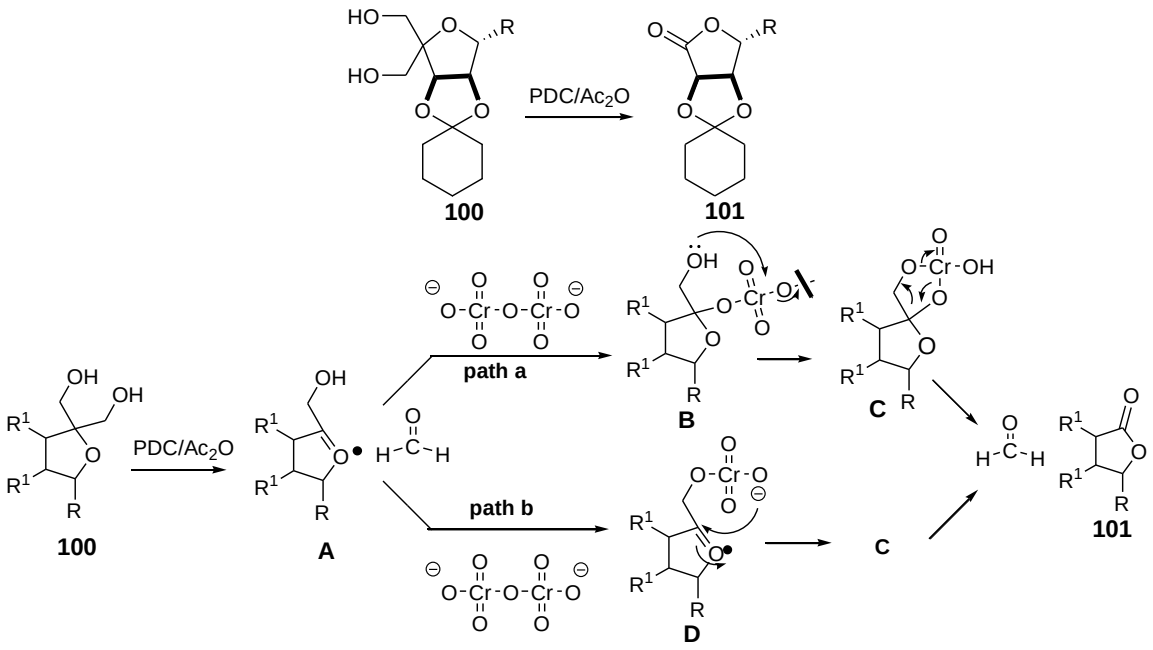
PDC is also useful for oxidizing aldehydes in aprotic media, giving rise to the corresponding acids.[27] However it requires a longer reaction time. During the synthesis of (±)dihydronepetalactone, the precursor **99** was synthesized from the corresponding aldehydes (**98**) by treating with PDC in DMF at 20^0C for 22 h (Scheme 1.47).[103]



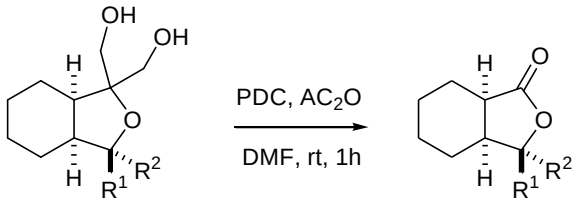
(Scheme 1.47)

PDC in presence of acetic anhydride (Ac_2O) as oxidizing system was used recently by Mahadevegowda and Khan for bis oxidative cleavage reaction of THF alcohols (**100**) to afford lactones (**101**).[104] A detailed plausible mechanism of the cleavage reaction was proposed (Scheme 1.48). The treatment of THFs with PDC in presence of acetic anhydride causes Cα-Cβ bond scission, driven by the generation of an oxonium intermediate A with expulsion of a formaldehyde. Intermediate **A** then reacts with another equivalent of dichromate in two possible path ways (a and b) to form intermediates **B** and **D** respectively. The reductive decomposition of cyclic chromate ester C through Cα-Cβ bond cleavage delivers c-lactones (**101**) with the

liberation of another equivalent of formaldehyde in both the pathways. This method was further extended for the oxidation of other THF diols (**102**) to afford lactones (**103**) in moderate yields (Scheme 1.49).[105]

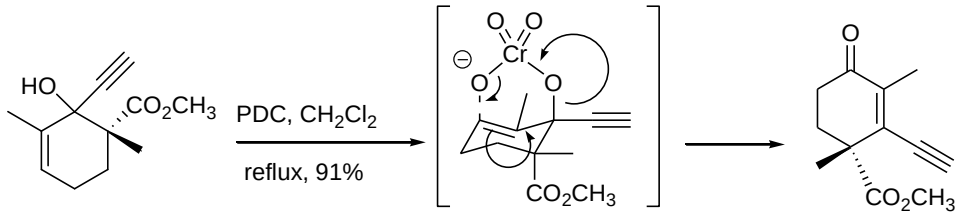


(Scheme 1.48)



(Scheme 1.49)

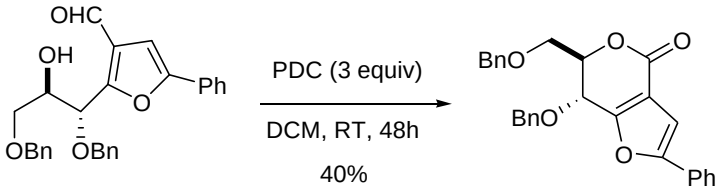
PDC was utilized for the oxidative allylic rearrangement of cycloalkenol (**104**) to afford the keto derivative **105** easily, despite a high degree of functionalization and steric interactions. It was assumed that the hydroxyl group is fixed in a pseudo-axial orientation in the transition state during [3,3]-sigmatropic rearrangement, thus minimizing the steric hindrance (Scheme 1.50). Any other orientation would cause severe steric interaction with the 3-methyl group.[106]



(+)-104 **(+)-105**

(Scheme 1.50)

Mal *et al.* synthesized furo[3,2-c]pyran-4-one upon PDC oxidation of chiral substituted furan without affecting the protecting groups and the stereochemistry of the adjacent carbon (Scheme 1.51). [107]



(Scheme 1.51)

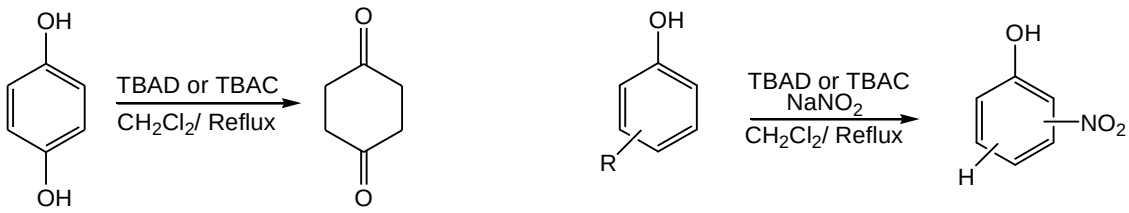
1.2.2 Alkylammonium halochromates and dichromates

With an aim to develop mild, lipopathic and chemoselective oxidizing system many alkylammonium ions are also used as counterions as the carrier of anion oxidants. Significant improvement has been achieved by the use of new oxidizing agents with tetralkylammonium ion like tetrahexylammonium, tetrabutylammonium tetrapropylammonium, tetraethylammonium, tetramethylammonium etc. as counter ions and chlorochromate, fluorochromate, bromochromate and dichromate as anionic oxidants.

Tetrabutylammonium ion has been extensively used due to its balanced amphiphilic characteristics. Various oxidants developed with tetrabuylammonium ion include tetrabutylammonium chlorochromate (TBACC),[108] fluorochromate (TBAFC),[109] chromate (TBAC),[110] bromochromate (TBABC).[111] TBAFC has been used for the effective and selective oxidation of alcohols, under mild conditions.[109]

TBABC and tetrapropylammonium bromochromate (TPABC)[111] oxidize alcohols to corresponding carbonyl compounds under mild conditions.

Pourali *et al.*[110] reported the conversion of oximes into the corresponding carbonyl compounds by using TBAC under homogeneous, aprotic and moderately acidic conditions. TBAC oxidizes hydroquinones to quinones in dichloromethane whereas in the presence of sodium nitrite it catalyzes the nitration process without affecting the phenolic hydroxyl group.[112] Similar reactivity has been observed for tetrabutylammonium dichromate (TBAD) under neutral aprotic conditions in CH_2Cl_2 (Scheme 1.52).



(Scheme 1.52)

Tetraethylammonium chlorochromate (TEACC)[113] is one of the versatile reagents for efficient and selective oxidation of organic substrates like crotonaldehyde in acetic acid.[114] TEACC was used by Tomar and Kumar for the oxidation of aldohexose like, D-mannose, D-fructose, D-glucose and D-galactose.[115] Oxidation kinetics of some α-hydroxy acids like glycolic, lactic, malic, and a few substituted mandelic acids to the corresponding carbonyl compounds with TEACC was studied by Swami *et al.* in DMSO medium (Scheme 1.53).[116] Similarly TEACC was also utilized for the selective oxidation of some lower oxyacids of phosphorus to the corresponding oxyacids with phosphorus in higher oxidation state in apolar medium (Scheme 1.53).[117]

(Scheme 1.53)

The conversion of diols and their monoethers to corresponding hydroxy carbonyl compounds,[118] organic sulphides to the sulfoxides,[119] aliphatic primary alcohols to aldehydes,[120] aliphatic aldehydes to carboxylic acids,[121] and deoximination of aldo- and keto-oximes[122] by TEACC in DMSO medium demonstrated the mildness and selectivity of this oxidant.

Tetramethylammonium fluorochromate (TMAFC) and chlorochromate (TMACC) prepared by the reaction of the corresponding quaternary ammonium salts with CrO_3 in a 1:1 molar ratio in acetonitrile medium constitute another class of lipopathic Cr(VI) oxidants.[123] TMACC was used by Hajipour *et al.* for oxidative deprotection of trimethylsilyl, tetrahydropyranyl ethers, ethylene acetals and ketals to the corresponding carbonyls [124] and selective oxidation of thiols to corresponding disulfides.[125]

A valuable addition to the prolific oxidant family is the trialkylammonium halochromates (R_3NH [CrO_3X]) (R= CH_3, C_2H_5, C_3H_7 and C_4H_9, X= Cl, F).[126] These reagents are of low cost, readily available and capable of oxidizing numerous organic substrates. Oxidative conversion of benzhydrols to the corresponding benzophenones by tributylammonium chlorochromate (TriBACC), alcohols to aldehydes or ketones, anthracene and phenanthrene to anthraquinone and phenanthraquinone by tripropylammonium fluorochromate (TriPAFC), thiols to corresponding disulfides by tripropylammonium chlorochromate (TriPACC) and TriPAFC are some note worthy applications of these reagents.[126]

Chemisorbed on alumina and silica, dimethylammonium chlorochromate (DMACC) was found to be effective for oxidation of alcohols, benzoins and regeneration of carbonyl compounds by oxidative cleavage of C=N under non-aqueous condition.[127-129] Similarly the effectiveness of N-methylbenzylammonium fluorochromate (MBAFC)[130] and N-ethylbenzylammonium fluorochromate (EBAFC)[131] was considerably increased upon its adsorption on silica gel. Many

functional groups are inert towards these oxidizing agents, including thiols, sulfides and phenols, enhancing the usefulness as chemoselective reagents for the synthesis of highly functionalized molecules. Regeneration of carbonyl compounds from their nitrogen containing derivatives (oximes, p-nitrophenylhydrazones, 4-phenylsemicarbazones and semicarbazones) was achieved using methylammonium chlorochromate adsorbed on silica gel (MCC/SiO_2) with good yields.[132]

Landini and Rolla synthesized bis-tetrabutylammonium dichromate (BTBAD) and used for the successful conversion of substituted benzyl bromides to the corresponding carbonyl compounds.[133] Under microwave conditions, deprotection of oximes by BTBAD was achieved by Murugan and Reddy.[134] By employing BTBAD, 1,4-diacylbenzenes was synthesized in good yield by Suhana and Srinivasan.[135] Tetramethylethylenediammonium dichromate (TMEDADC) obtained from CrO_3 and TMEDA was utilized for selective oxidation of benzylic and allylic alcohols.[136]

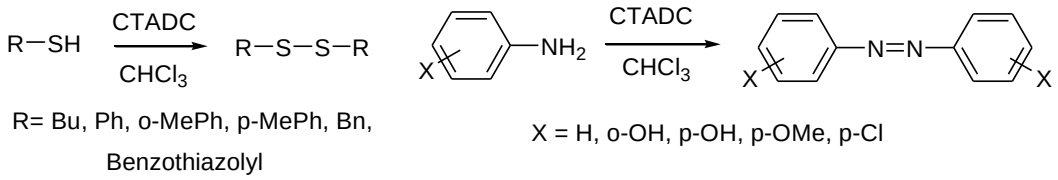
Cetyltrimethylammonium dichromate (CTADC) was synthesized by Patel *et al.* by a simple ion exchange method from cetyltrimethylammonium bromide (CTAB) and potassium dichromate (Scheme 1.54).[137] CTADC is insoluble in water and soluble in most of the organic solvents. It is found to be stable in these solvents at reflux temperature and for an appreciable time period (>24 h). On water surface, it forms condensed monolayer and assumes an area of 51 $A°^2$/ molecule at a temperature of 298 K.[138]

$$2C_{16}H_{33}N^{+}(CH_3)_3Br + K_2Cr_2O_7 \rightarrow [C_{16}H_{33}N^{+}(CH_3)_3]_2Cr_2O_7 + 2KBr$$

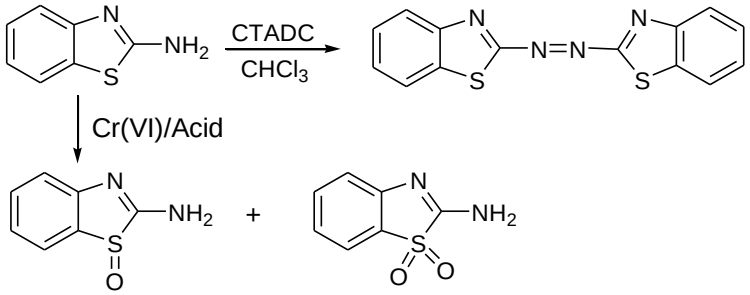
(Scheme 1.54)

CTADC has shown its effectiveness in the oxidation of various functional groups like alcohols, aldehydes, hydroxyquinones, cinnamic acid etc in chloroform or DCM under mild reaction conditions.[137] The oxidized products of alcohols and hydroxyquinones are found to be the corresponding carbonyl compounds and

benzoquinones, respectively. CTADC converts cinnamic acid to benzoic acid and aromatic aldehydes to benzoic acids in nonpolar medium. The reagent oxidizes amino and mercapto groups to give the corresponding diazo and disulfides respectively through dehydrogenation process (Scheme 1.55).[139] This strategy of selective oxidation of amino compounds by CTADC was successfully demonstrated in the oxidation of 2-aminobenzothiazole to the corresponding diazo product selectively instead of expected sulfur oxidized products (sulfoxide or sulfone) (Scheme 1.56).



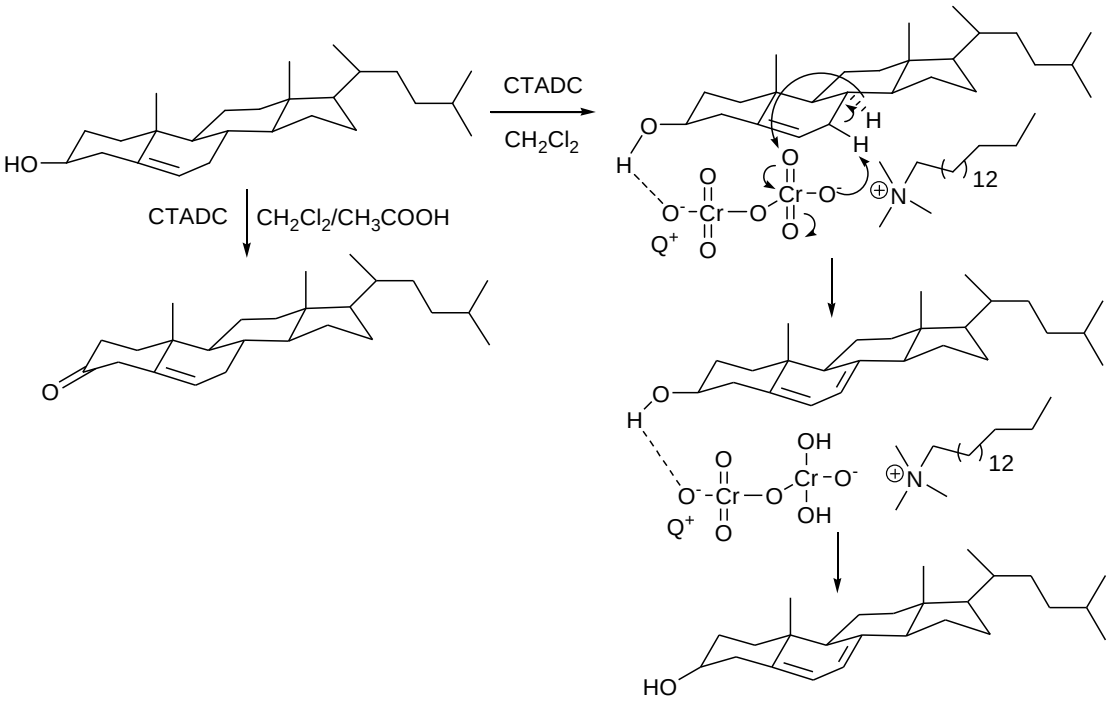
(Scheme 1.55)



(Scheme 1.56)

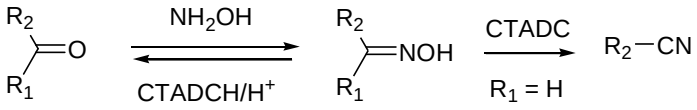
Oxidation of cholesterol by CTADC in refluxing DCM afforded 7-dehydrocholesterol as a result of the introduction of a double bond by dehydrogenation process. The –OH group at C3 remain unaltered. The proposed remote functionalization mechanism suggests the initiation of the reaction through an association of the 3-OH group with the chromate ion of CTADC and a secondary overlap of π-orbitals of cholesterol at the C5-C6 position with that of Cr=O bond, which assist the system in achieving proper orientation. Subsequent reaction takes place at an equidistant site of the active centre of the reagent at the cholesterol nucleus (Scheme 1.57).[140] In the presence of acetic acid however the expected 5-cholesten-3-one was isolated as the major product. The difference in the reactivity in the presence

of acetic acid is proposed to be primarily due to the decrease in the contactness between CTA cation and dichromate anion.[138]



(Scheme 1.57)

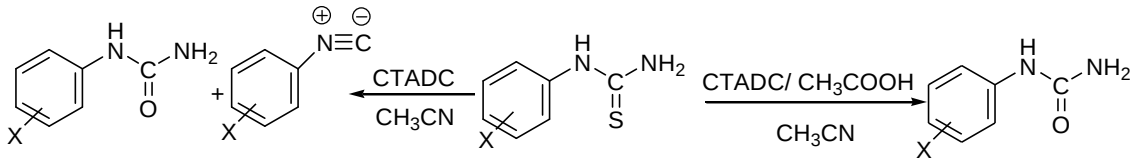
The difference in reactivity of CTADC in terms of product formation in the presence of acetic acid and in the absence of acetic acid was also observed in the oxidation of oximes (Scheme 1.58).[141-142] Easy deprotection of oximes was achieved by CTADC in the presence of a trace amount of acetic acid in dichloromethane. However, when the above reaction was performed in the absence of acetic acid, the corresponding nitrile derivatives was obtained from aldoximes while ketoximes was found to be unreacted.



R_2 = aryl group
R_1 = H, Me, $C_6H_5CH(OH)$
R_1CR_2 = cyclohexane

(Scheme 1.58)

In order to explore the chemoselectivity of CTADC, a series of substituted phenylthioureas have been oxidized in organic medium.[143-144] Oxidation of phenylthiourea by CTADC in acetonitrile without any acid afforded phenyl isonitrile, but in presence of acetic acid, the product was found to be phenylurea. In case of substituted phenylthioureas, the products were corresponding ureas and isonitriles in neutral condition and corresponding ureas only in acidic condition (**Scheme 1.59**). The mildness and chemoselectivity of CTADC have also been observed in the oxidation of benzyl alcohol and simvastatin.[145-146]



(Scheme 1.59)

1.2.3 Phosphonium and tellurium chromates and dichromates

Some chromates and dichromates with other than nitrogen oniums like phosphonium and telluronium were synthesized to deal with specific oxidation reactions (Table 1.1).

Table 1.1 Examples of some chromates and dichromates containing phosphonium and tellurium counterions.

Sl. No.	Reagent	Investigation
1	Benzyltriphenyl phosphonium chlorochromate (BTPPCC)	Selective oxidation of benzyl alcohol in presence of phenyl ethanol, benzhydrol or methyl phenyl sulfide.[147] Oxidation of sulfides to corresponding sulfoxides.[148]
2	Butyltriphenyl phosphonium chlorochromate (BuTPPCC)	Oxidation of alcohols to carbonyl compounds.[149]
3	Butyltriphenylphosponium dichromate (BTPPD)	Oxidation of alcohols to carbonyl compounds, thiones to corresponding carbonyl compounds by microwave irradiation without any solvent, thiols to corresponding disulfides under microwave irradiation and sulfides to sulfoxides and sulfones in presence of aluminium chloride in solution and under microwave irradiation, aldehydes to carboxylic acid, amino acids to the corresponding aldimines, diols to hydroxyl carbonyls.[150-157]
4	Tetrabutylphosphonium dichromate (TBPDC)	aromatization of 1,4-dihydropyridines to corresponding pyridine derivatives in acetonitrile and also under microwave irradiation.[158]
5	Triphenylmethylphosphonium dichromate (MTPPD)	solid phase oxidation of benzylic alcohols to the corresponding aldehydes and ketones under solvent-free conditions with high chemoselectivity.[159]
6	Benzyldimethyltelluronium dichromate (BDMTDC)	Selective oxidation of benzylic and allylic –OH group in presence of other primary or secondary –OH group.[160]

1.3 OXIDATION OF ORGANIC SUBSTRATES BY LIPOPATHIC Mn(VII)

Permanganate has been widely used as a strong, easily handled, readily available and versatile oxidant that reacts with alcohols, alkenes, aldehydes, saturated C–H bonds, steroids and other functionalities. [161-166] The lack of selectivity of permanganate is due, at least in part, to its ability to react readily by either one- or two electron pathways, and its conversion into even stronger oxidants such as MnO_3^+. The reaction pathways are influenced by solvent, pH, solubility, substrate and other variables, thus complicating the mechanistic understanding.

Before the discovery of lipopathic Mn(VII) oxidants, permanganate oxidations of organic substances were performed in aqueous media with organic co-solvents, in which potassium permanganate shows an appreciable solubility and inertness. The co-solvents mostly used are *t*-butanol, acetone, pyridine, acetic acid, acetic anhydride, trifluoroacetic acid etc. The use of organic solvents allows the substrate and solvent to be in the same phase and avoids some of the complications of aqueous permanganate reactions, such as decomposition at high pH,[167] autocatalysis at low pH,[168] involvement of water in the rate determining step[169] and limited solubility of the organic substrates of interest.[170]

Phase transfer catalysis (PTC) has been recognized as one of the versatile techniques for permanganate mediated oxidation of water insoluble organic substrates. Gibson and Hosking were the first to report on the phase transfer catalytic permanganate oxidation of water insoluble substrates in organic media by triphenylmethylarsonium permanganate pairing the permanganate ion with a quaternary lipophilic cation.[171] Purple benzene was prepared by using dicyclohexyl-18-crown-6 as PTC from solid potassium permanganate by Sam and Simmons and subsequently used for many organic reactions.[172] Herriott and Picker used different tetraalkyl ammonium salts to extract permanganate ion from aqueous solution for oxidation reactions.[173]

For the use of Mn(VII) as the oxidant for organic substrates in organic solvents, crown ethers and onium ions as the carrier of oxidants have wide applications as phase transfer catalysts. An extensive review in this regard has been presented by Dash *et al.*[8] In nonpolar solvents, due to the amphipathic nature of the tetraalkylammonium ions, quarternary ammonium permanganates are effective reagents for the oxidation of organic substrates.[174-176] However, many organic solvents are highly sensitive to the oxidation potential of Mn(VII) and thus have limited use as solvents for oxidation reaction.[2, 177] Further, decomposition of some quaternary salts, self oxidation and instability of most of these Mn(VII) oxidants in organic solvents limit their uses as lipopathic oxidants.[178-180] Some significant applications of these oxidants have been presented here.

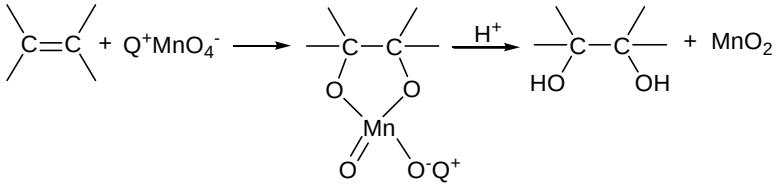
Tetraalkylammonium permanganates are synthesized by simple ion exchange method by mixing tetraalkylammonium bromides and potassium permanganate in aqueous medium. These reagents are excellent phase-transfer oxidants for organic substrates in completely non-polar organic solvents, such as benzene, dichloromethane, chloroform, carbon tetrachloride, toluene, etc., and in completely anhydrous conditions. Quaternary ammonium salts in aqueous medium dissociate to constituting ions, while in organic solvents these salts exist in ion pairs.[175, 181-185] The probability of salts existing as ion pairs is inversely proportional to the distance between the centres of the two ions and the dielectric constant of the solvents. The closeness between ion pairs was found to influence the rate of reaction. As an example, the rate constants for the oxidation of methyl cinnamate by methyltri-n-octylammonium permanganate are greater than that for tetra-n-octylammonium permanganate, because the former allows a greater penetration of the anion into the structure of the cation due to lesser steric hindrance. When styrene derivatives were oxidized by quaternary ammonium or phosphonium permanganates in a polar organic solvent, such as acetone, the substituents and counterions have little or no effect on the rate of reaction.[182] In less polar solvents, such as DCM or toluene, the rates of the

reaction are, however, dependent upon the nature of the quaternary ammonium or phosphonium ions. Thus it can be concluded that, quaternary ammonium permanganates exist as solvent-separated ion pairs in dipolar aprotic solvents like acetone, while they exist as tight ion pairs in apolar solvents like toluene and dichloromethane.

Weber and Shepherd[186] oxidized cyclohexene, cis-cyclooctene and trans-cyclooctene to vicinal *cis*-diol stereospecifically by cold, dilute alkaline potassium permanganate in the presence of a catalytic quantity of benzyltriethylammonium chloride as PTC in water-dichloromethane mixture. Subsequently, Ogino and Mochizuki[187] reported that $KMnO_4$ solubilized in dichloromethane in the presence of an equimolar amount of benzyltriethylammonium chloride readily oxidizes alkenes to either 1,2-diols or aldehydes depending upon pH of the medium without any over-oxidation.

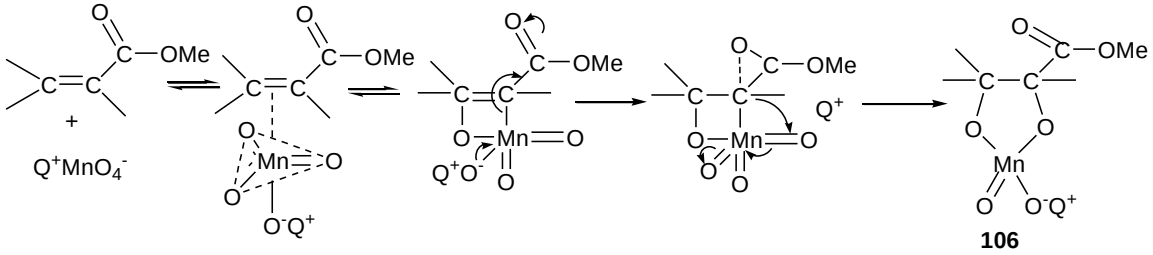
In an oxidation reaction of methyl (E)-cinnamate with quaternary ammonium permanganates in dichloromethane solutions, Lee and Brown[188] proposed that the counter ion has a substantial effect on the rate of reaction. The rate of reaction is fastest for those in which inter ionic distance in the quaternary ammonium ion pair is minimum. Hence, smaller cations have grater effects on the reactivity and promote a faster reaction.

Lee and Perez-Benito[189] have used methyltributylammonium permanganate (MTBAP) for the oxidation of 1-tetradecene in dichloromethane. Manganate(V) diesters was proposed to be the intermediate which on hydrolysis afforded *cis*-diol and colloidal manganese dioxide (MnO_2) (Scheme 1.60). The colloidal MnO_2 formed during the reaction was found to catalyze the reaction by providing a surface on which the catalyzed reaction takes place.



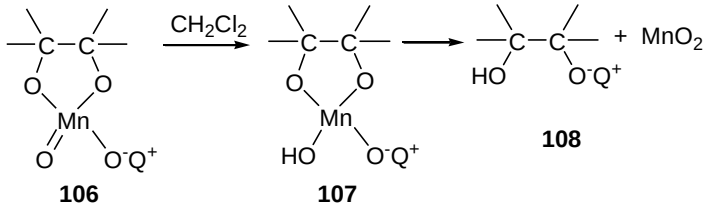
(Scheme 1.60)

Tetrabutylammonium permanganate (TBAP) was used as a phase transferring oxidant for the oxidation of substituted methyl cinnamates.[189] Positive ρ (reaction constant) values confirmed higher electron density in the transition state than the ground state. The numerical value of reaction constants (ρ) was found to be greater in acetone (ρ=1.43) than that of dichloromethane (ρ=0.95) which further supported the existence of tight ion pair in nonpolar solvents. Involvement of a rate limiting heterolytic cleavage of the carbon–manganese bond to give an enolate-like transition state was proposed (Scheme 1.61). The proximity of the quaternary ammonium ion through the formation of tight ion pair was proposed to increase the stability of the transition state in non-polar solvents. On the other hand, in dipolar aprotic solvents, quaternary ammonium ion contributes less towards the stability of the transition state due to the formation of solvent separated ion pairs. The hypomanganate (**106**) being a very reactive intermediate, rapidly converted to a manganese(IV) cyclic diester **107** via a one-electron reduction, possibly by abstraction of a hydrogen atom from a molecule of solvent (Scheme 1.62). **107** subsequently decomposed to a diol anion (**108**) and MnO_2.[189]



106

(**Scheme1.61)**



(Scheme 1.62)

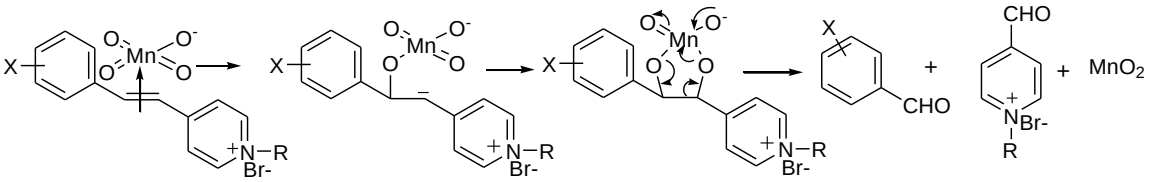
Oxidation of unsaturated carboxylic acids was carried out using MTBAP in non-aqueous solvents.[190] The reaction was found to differ in several ways, both from the corresponding aqueous-phase oxidations[191] and from the oxidation of unsaturated esters.[192] A Mn(III) species was found to be the final product of the reduction of permanganate by unsaturated acids in dichloromethane. The catalytic activity was proposed to be due to the formation of a powerful oxidant $HMnO_4$ (Scheme 1.63).

(Scheme 1.63)

An important addition to the lipopathic Mn(VII) oxidant family is the cetyltrimethylammonium permanganate (CTAP) synthesized by adding aqueous solution of CTAB to the aqueous solution of potassium permanganate ($KMnO_4$). It is sparingly soluble in aqueous medium but highly soluble in nonpolar solvents such as chloroform, dichloromethane etc.[193]

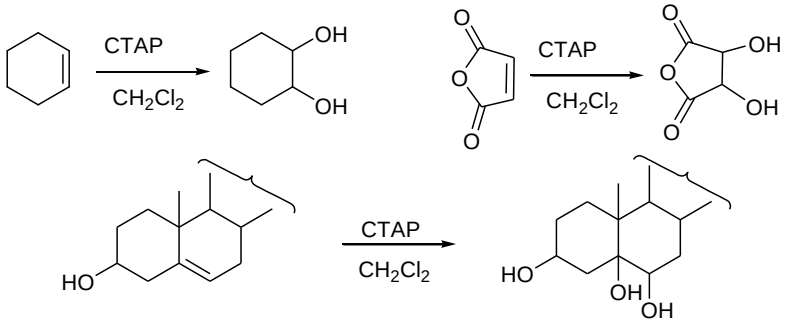
In order to explore the chemoselectivity of lipopathic Mn(VII), Mishra e*t al.* have used CTAP for the oxidation of some monochromophoric styrylpyridinium dyes in chloroform medium and compared the results with the oxidation of the same substrate using $KMnO_4$ in acidic medium.[193] Due to the formation of tight ion pair in non-polar medium and also due to the charge on the substrate, both hydrophobic interaction and electrostatic effect bring the reactant and the substrate into close proximity, thereby facilitating the reaction. A plausible mechanism for the cleavage of double bond to form carbonyl products was proposed based on experimental evidences (Scheme 1.64). A similar mechanistic pathway leading to oxidative

cleavage of *trans*-double bond was also proposed for the oxidation of some substituted alkyl cinnamates by CTAP in chloroform medium.[194] The proposal of negatively charged transition state was supported by the rate retarding effect of electron-donating and rate accelerating effect of electron-withdrawing substituents.

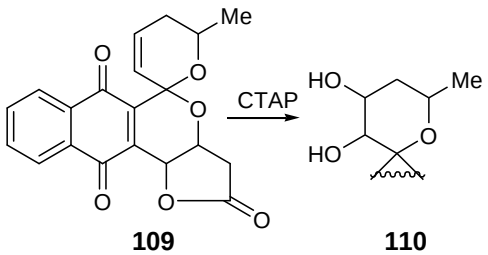


(Scheme 1.64)

During the oxidation of styrylpyridinium dyes and alkyl cinnamates, which contain trans-double bonds, bond breaking was observed, leading to the formation of carbonyl compounds selectively. In case of some compounds containing cis-double bonds, e.g., cyclohexene, maleic anhydride and cholesterol, oxidation products were found to be corresponding diols (Schemes 1.65).[195] The strategy of oxidation of cis-double bond by CTAP was applied successfully for the *cis*-dihydroxylation of the spiro fused dihydropyran ring of **109** to afford an analog of antibiotic griseusin A(**110)** (Scheme 1.66).[196]



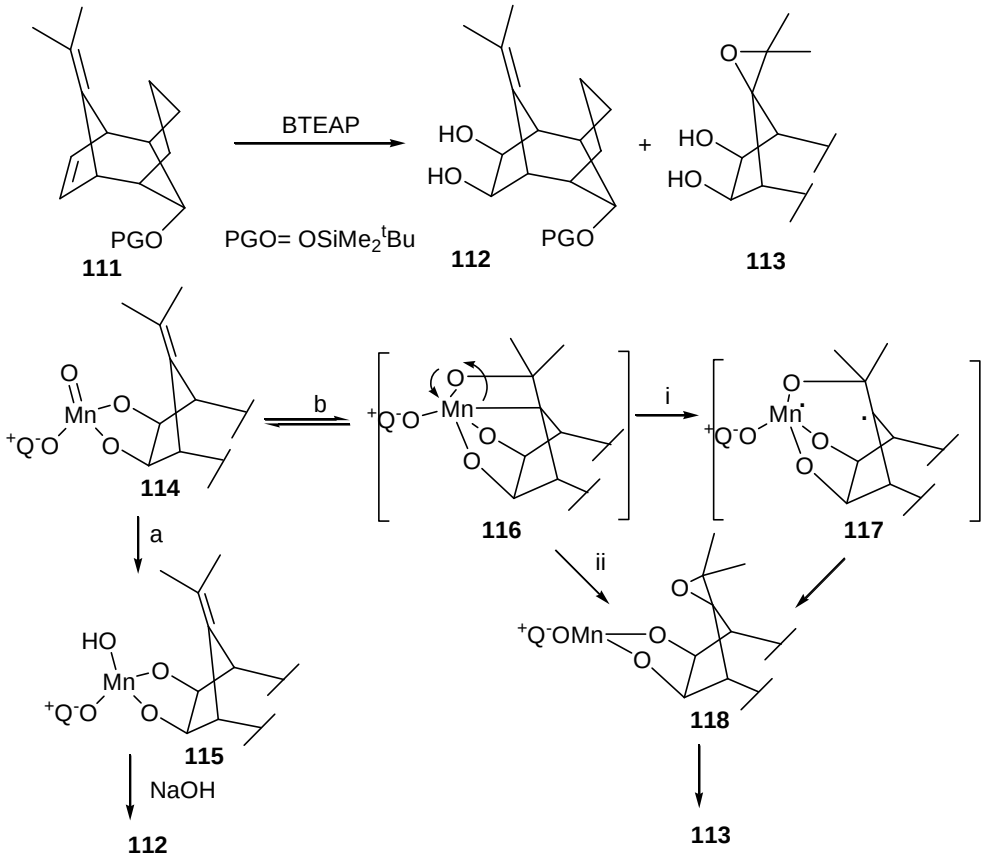
(Scheme 1.65)



109 **110**

(**Scheme 1.66**)

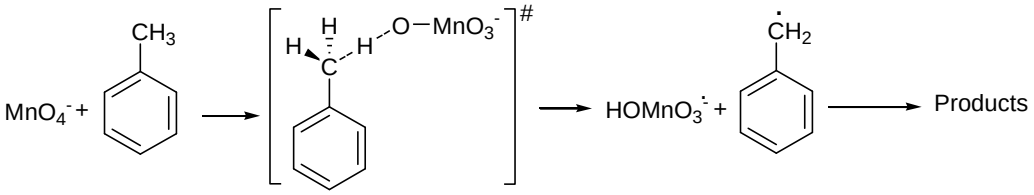
Treatment of a tricyclic rigid diene (**111**) with one equivalent of benzyltriethylammonium permanganate (BTEAP) at low temperature (-50^{o}C) followed by quenching with aqueous sodium hydroxide afforded the diol **112 (**70% yield) together with the diol epoxide **113** (20%).[197] With increase in the temperature, yield of diol decreases with the formation of dialdehyde. Formation of **112** and **113** has been proposed as depicted in Scheme 1.67. The cyclic manganese (V) diester **114**, formed by the attack of the permanganate on the most reactive double bond, decomposes into diol **112** or epoxy-diol **113** through two different reduction processes. One electron reduction by reaction with the solvent to give the Mn(IV) diester **116** through path 'a' resulted in the diol **112** while an intramolecular oxygen transfer to the other double bond which lies in close proximity to the manganese centre through path 'b' yielded epoxy-diol **113.**



(**Scheme 1.67)**

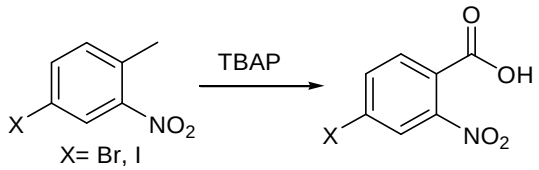
Oxidation of C-H bonds by permanganate is an important class of organic transformations. Alkylammonium permanganates have been found to oxidize C-H bonds in organic solvents.[174, 198-199] Tetraethyl, tetrabutyl and benzyltriethylammonium permanganate and methyltriphenylphosphonium permanganate are found to be about equally effective as oxidants for the conversion of alkanes into alcohol and ketones.

In the oxidation of aryl alkanes such as toluene, ethylbenzene, diphenylmethane, triphenylmethane, 9,10-dihydroanthracene, xanthenes and fluorene by TBAP, toluene is oxidized to benzoic acid and a small amount of benzaldehyde where as other substrates are converted to carbonyl compounds and/or dehydrogenated products.[200] The reduced manganese product of all of these reactions is colloidal MnO_2. Abstraction of a hydrogen atom by permanganate was proposed to be the first step of these reactions facilitated due to the formation of strong O-H bond (Scheme 1.68).[169,201]



(Scheme 1.68)

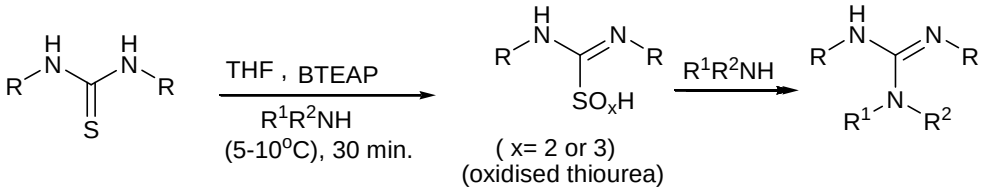
Oxidation of 4-halo-2-nitrotoluene with TBAP in pyridine was found to be an efficient method to synthesize 4-halo-2-nitrobenzoic acid (Scheme 1.69).[202] To control the vigorous exothermic reaction and to do the reaction in a multi-gram scale, control feeding of cold TBAP solution into the reaction mixture at 60°C was performed and thus the initiation process was managed.



(Scheme 1.69)

2,4-Dimethylnitrobenzene (2,4-DMNB) was oxidized to 3-methyl-4-nitrobenzoic acid with potassium permanganate under heterogeneous conditions in the presence of a phase transfer catalyst, tetrabutylammonium bromide (TBAB). The reaction was carried out at 95 °C for 1 h with the ratio of n(2,4-DMNB): n(KMnO4): n(TBAB) = 1 : 2.16 : 0.07 and 3-methyl-4-nitrobenzoic acid was obtained in 41% yield. In the absence of PTC 4-nitro-1,3-benzenedicarboxylic acid was obtained as the only product. Thus it can be concluded that presence of TBAB as PTC brings mildness, regioselectivity and chemoselectivity in the oxidation reaction through *in situ* formation of TBAP.[203]

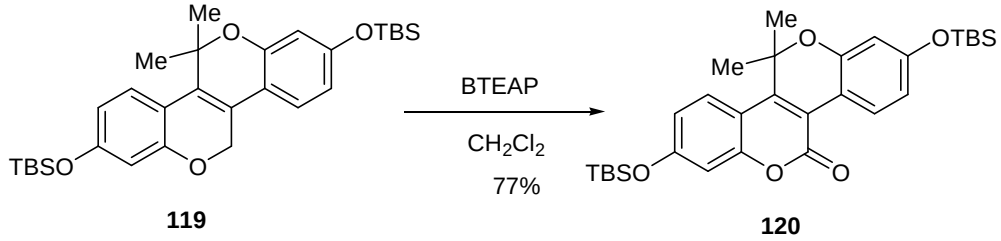
Srinivasan and Ramadas[204] synthesized trisubstituted guanidines in excellent yields from 1,3-diarylthioureas using quaternaryammonium permanganates in the presence of an amine in THF (Scheme 1.70). This proposed scheme was preferred since the sulfonyl group is reported to be displaced about 15 times faster than the corresponding *S*-alkylated species in the case of monosubstituted thioureas.[205] From a comparative study using BTEAP, CTAP and TBAP on several thioureas and amines it was found that BTEAP is better than the other two because of its stability, shock-resistant and decomposition above 100°C. CTAP furnished poor results and the product isolation was rendered difficult due to foaming during the follow-up action in aqueous medium.



(**Scheme 1.70)**

Jain *et al.* have successfully utilized BTEAP for the oxidation of the allylic methylene group of **119** in dichloromethane medium and isolated the desired lactone **120** in 77% yield (Scheme 1.71).[206] Jones reagent, MnO_2, and RuO_4 failed to produce the desired allylic oxidation product. Oxidation via selenium dioxide, PCC, and KO_2

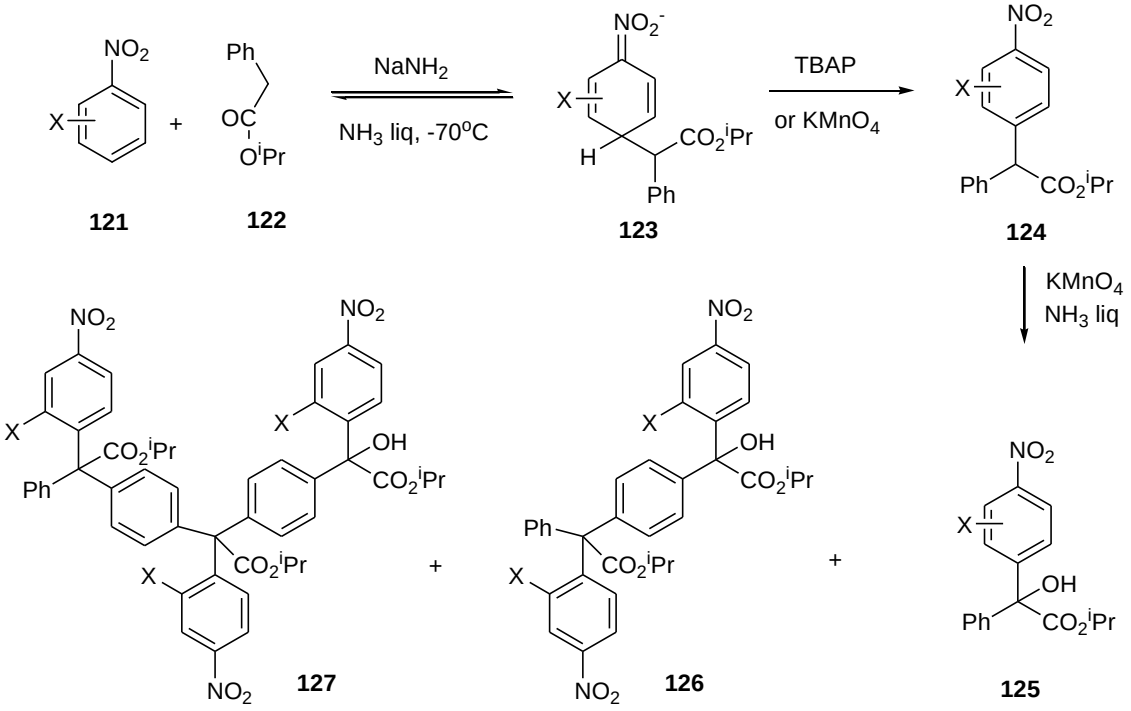
also proved unsuccessful. A protocol originally developed by Paquette *et al.*[207] using a mixture of PCC (5 equiv), chromium trioxide (5 equiv), and 3,5-dimethylpyrazole (10 equiv) was found to be successful and yielded 11,11-dimethylbisbenzopyran lactone **120** in moderate yield (54%). This shows the selectivity and efficiency of BTEAP for allylic oxidation process.



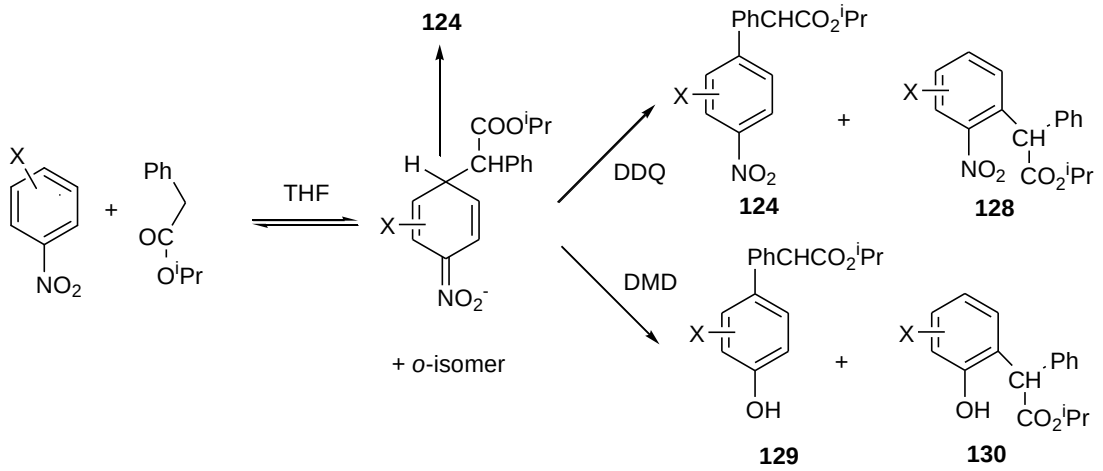
119 **120**

(Scheme 1.71)

Makosza *et al.*[208] used TBAP in the oxidative nucleophilic substitution of hydrogen (ONSH) in nitroarenes with carbanion of isopropyl phenyl acetate. TBAP was found to be more suitable and advantageous than other oxidizing systems such as $KMnO_4$, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), dimethyl dioxirane (DMD)etc (Scheme 1.72 and 1.73). Secondary carbanions of isopropyl phenyl acetate (**122**) add to nitroarenes (**121**) at ortho and para to the nitro group. The produced anionic σ^H adducts (**123**) were then oxidized to a variety of products depending on the oxidizing agent and medium of the reaction. Permanganate anions oxidize only σ^H adducts para to the nitro group, the final outcome depends on the conditions. The reaction carried out in liquid ammonia and $KMnO_4$ as oxidant gives iso-propyl-α-hydroxy-α-nitroarylphenylacetates (**125)** formed via hydroxylation of the initial ONSH products (**124**). In some cases additionally dimeric (**126**), trimeric (**127**) and tetrameric products were also found. On the other hand, in THF ONSH product (**124**) was found to be the only product through selective oxidation of σ^H adducts para to the nitro group. No over oxidation or side reactions were observed in this case. DDQ oxidized both ortho and para σ^H adducts giving rise to mixtures of isomeric ONSH products (**124** and **128**). Oxidation by DMD gave isomeric substituted phenols (**129** and **130**).



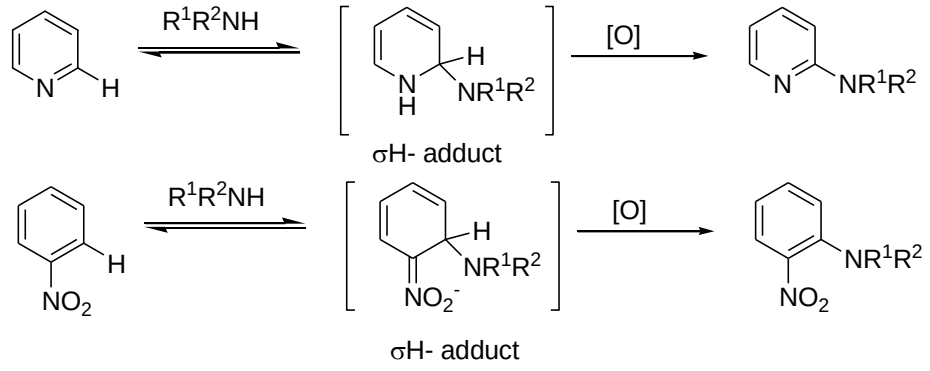
(Scheme 1.72)



(Scheme 1.73)

Verbeeck *et al.*[209] have studied the mechanism of oxidative alkylamination of electron-deficient (hetero) aromatic compounds, via the oxidative nucleophilic substitution of hydrogen (ONSH) using $AgPy_2MnO_4$, $KMnO_4$, $AgNO_3/KMnO_4$, $TBACl/KMnO_4$ or TBAP as oxidant. On the basis of competitive kinetic isotope effect (KIE) experiments, oxidation step of the intermediate σ^H-adduct was proved to be the rate-limiting step of oxidative alkylaminations (Scheme 1.74). In those cases

where the reaction with $KMnO_4$ gave a low conversion and yield due to solubility problem, TBAP was found to a better alternative for $TBACl/KMnO_4$ and expensive $AgPy_2MnO_4$ as it involved cheaper and more environmentally friendly reagents.



(Scheme 1.74)

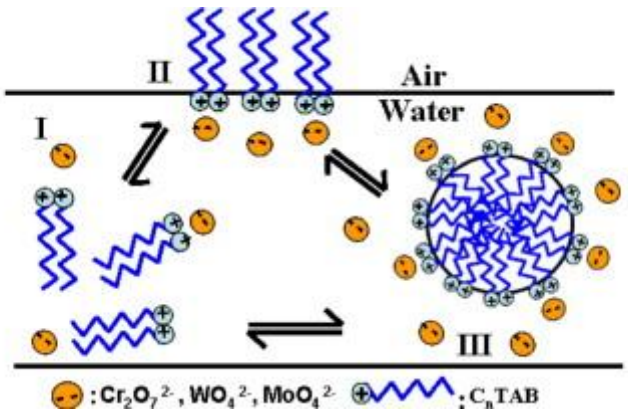
1.4 SELF AGGREGATION OF LIPOPATHIC OXIDANTS CONTAINING LONG CHAIN ONIUM IONS

Lipopathic oxidants derived from long chain cationic onium ion and anionic counterions are known to have surface active properties and form various structured aggregated species like micelles, vesicles, liquid crystals, monolayers, bilayers, etc., and have drawn the attention of scientists for their multiple application potentials.

Mishra and coworkers have studied the surface active properties of lipopathic oxidants CTADC, CTAP and CTACN in different medium. All these three lipopathic oxidants form condensed monolayer at air-water interface.[138] From the isotherms of surface pressure versus area of CTADC, CTAP and CTACN the anchoring area of each oxidant on air-water interface were determined to be 51, 22 and 45 $Å^2$ respectively when spreading as Langmuir monolayer. The area/molecule of these oxidants with a common amphiphilic cationic CTA counterion depends largely on the size of the anionic counterpart indicating the existence of contact ionpair between the respective ions.[138] Further, during the investigation of oxidation kinetics of some organic substrates in nonaqueous medium, the formation of reverse micellar and

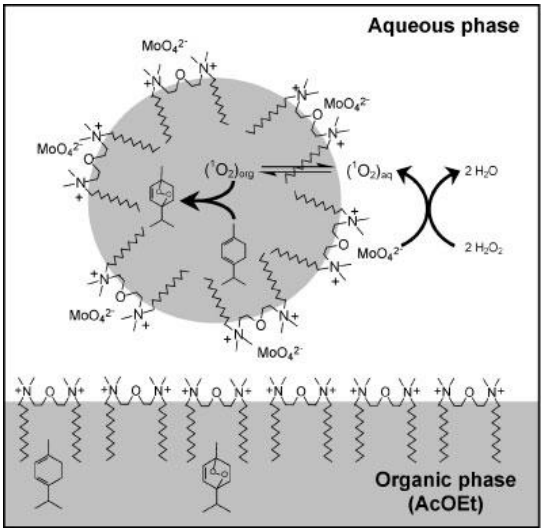
mixed reverse micellar type aggregates of CTADC and CTAP has been encountered.[10, 140,142,145,146, 193,194]

Mukherjee et al.[11] have synthesized some amphiphilic oxidants of dichromate, tungstate and molybdate of the type $[C_nH_{2n+1}(CH_3)_3N^+]_2\ X^{2-}$ (n = 10, 12, 14, 16 and 18 and X= $Cr_2O_7^{2-}$, MoO_4^{2-} and WO_4^{2-}) and investigated their bulk and interfacial properties in aqueous medium and at air-water interfaces (Scheme 1.75). Formation of layered structures in solid state was supported by X-ray diffraction (XRD) measurements. The phase transitions of the compounds were studied by thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis. The dichromate amphiphiles were found to be poorly soluble in water whereas the tungstates and molybdates were found to be water soluble. The authors have studied the interfacial behavior of these dichromate amphiphiles at air-water interface. The Langmuir monolayers were found to be compressible and the higher homologues collapsed at higher states of compression. Cconductometry, tensiometry and calorimetry distinctly supported the formation of micelles for tungstate and molybdate complexes. The size and the zeta potential of the micelles of synthesized tungstate and molybdate complexes were found to increase with the increase in length of the hydrophobic tail of the complexes.



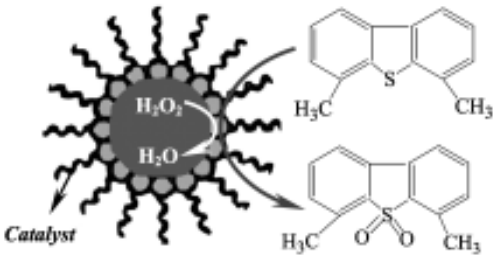
(Scheme 1.75)

A novel diammonium Gemini surfactant phase transfer catalyst, diethyl-ether-α,ω-bis-dimethyldodecylammonium molybdate (12-EO-12-Mo), containing C12 alkyl chain was found to enable the dark singlet oxygenation of organic substrates such as α-terpinene, β-citronellol etc. by chemically generated $^1O_2$.[17] 12-EO-12-Mo provides a simple reaction medium with only three components for the preparative peroxidation of hydrophobic substrates and catalyze the reaction through possible formation of aggregated structure in aqueous medium or in aqueous-organic biphasic medium as depicted in Scheme 1.76.



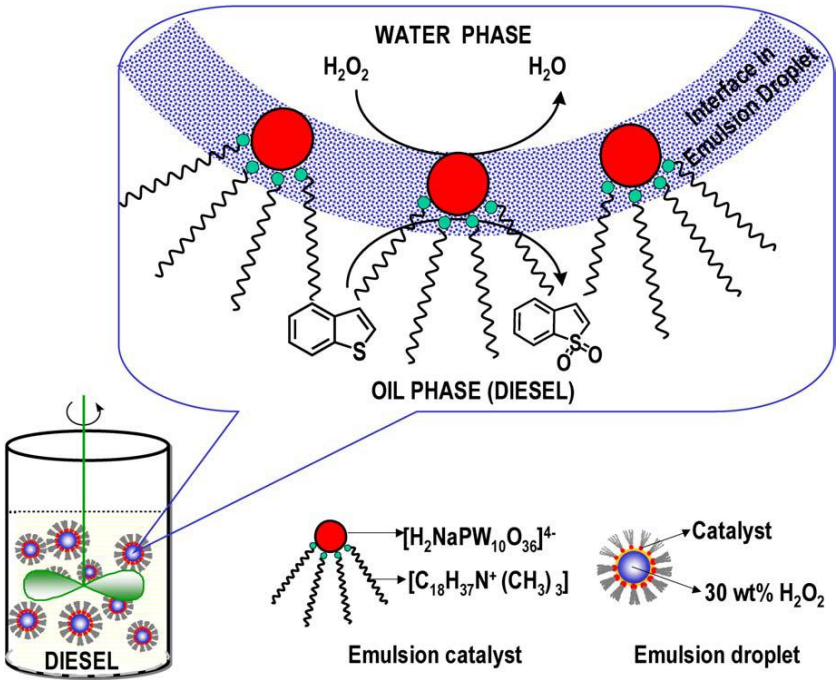
(Scheme 1.76)

Polyoxometalates (POMs), such as a quaternary ammonium polytungsto-phosphate catalyst when assembled at the interface of the emulsion droplets, catalyze the oxidation of sulfur-containing compounds presented in fuel oils.[210-213] The lipopathic catalyst $[(C_{18}H_{37})_2N^+(CH_3)_2]_3[PW_{12}O_{40}]$ containing C18 carbon chain when assembled in emulsion in diesel, can selectively oxidize 4,6-dimethyldibenzothiophene-like compounds to sulfones with stoichiometric amounts of H_2O_2 under mild conditions (Scheme 1.77).[15] The sulfones can then be readily separated from the diesel by using extraction methods and the catalyst can be recycled. The sulfur level can be lowered from about 500 ppm to 0.1 ppm without changing the properties of the diesel.



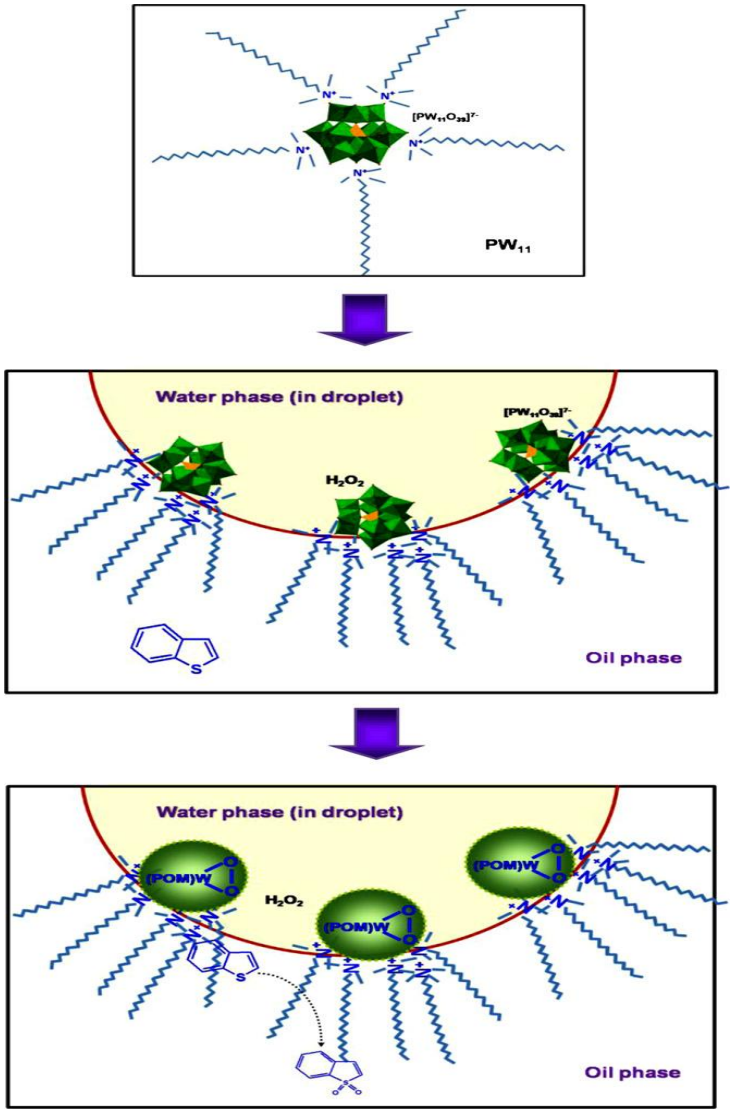
(Scheme 1.77)

An ultra-deep desulfurization process for diesel, based on similar catalytic oxidation with the catalyst $[C_{18}H_{37}N(CH_3)_3]_4[H_2NaPW_{10}O_{36}]$ assembled in emulsion droplets followed by extraction, was developed by Li and his coworkers.[14] The catalyst assembled in emulsion droplets was found to have very high intrinsic catalytic activity and selectively oxidize all kinds of sulfur-containing compounds present in model and actual diesel using an approximately stoichiometric amount of H_2O_2 as an oxidant under mild conditions. The sulfones formed in the diesel can be removed by a polar extractant. A schematic diagram as described by Li and his coworkers of this process has been presented in scheme 1.78.



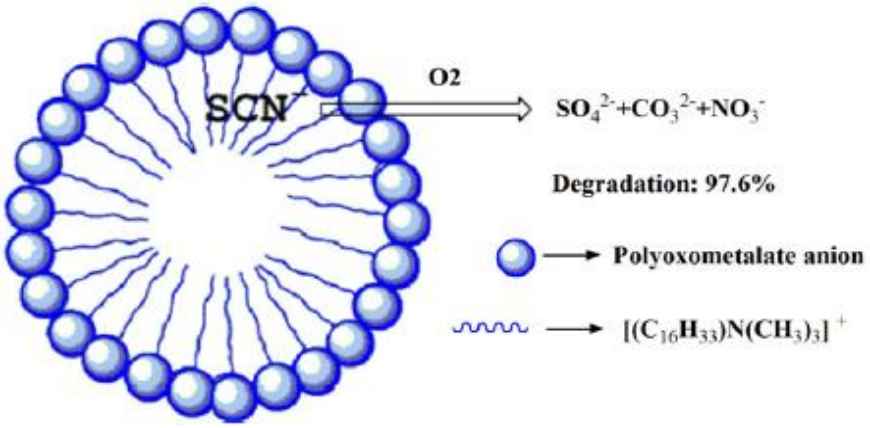
(Scheme 1.78)

Zhang *et al.*[13] developed a quarternary ammonium polytungstophosphate $[C_{18}H_{37}N(CH_3)_3]_5Na_2[PW_{11}O_{39}]$ (PW_{11}) with lacunary Keggin-structures as a selective catalyst for oxidative desulfurization. The amphiphilic catalyst PW_{11} was found to assemble at the interface of the emulsion droplets (Scheme 1.79) and exhibit high catalytic activity towards the oxidation of BT to corresponding sulfone under mild conditions with H_2O_2 as oxidant in emulsion system.



(Scheme 1.79)

Recently, oxidation of SCN^- by O_2 as the oxidant in a micellar system, where amphiphilic catalysts $[CH_3(CH_2)_{15}N(CH_3)_3]_5[PW_{11}(TiO_2)O_{39}]$ (**131**), $[CH_3(CH_2)_{15}N(CH_3)_3]_7$ $[PW_{10}(TiO_2)_2O_{38}]$ (**132**), and $[CH_3(CH_2)_{15}N(CH_3)_3]_9$ $[PW_9(TiO_2)_3O_{37}]$ (**133**) act as the surfactant and the catalysts, leading to simple inorganic species SO_4^{2-}, HCO_3^- and NO_3^- under extremely mild conditions was reported by Wei *et a.l* (Scheme 1. 80). These catalysts exhibit high efficiency of oxidation, ease of separation, long lifetime, and regenerability. The number of peroxo-titanium influences the catalytic activity, which shows the active range: **131**<**133**<**132**. This result could provide information on designing catalyst in oxidation reactions.[12]



(Scheme 1.80)

Similarly, many polyoxometalates containing long chain quaternary ammonium ions are synthesized and used as catalyst along with H_2O_2 as stoichiometic oxidant for green and selective oxidation of many organic functional groups.[214-219] The catalytic activity is mostly due to their ability to aggregate on emulsion droplets or at aqueous-organic interfaces.

1.5 SCOPE OF THE WORK

Literature studies, as mentioned above, envisage wide applications of anionic oxidants with quaternary ammonium as the counter ion in oxidation reactions of substrates in both aqueous and nonaqueous medium. The interactions of oxidants, quaternary ammonium ions and the solvents in a reaction system lead to a differently behaving reaction condition, wherein, a strong oxidant can be mild, and a weak oxidant can have enhanced oxidation potential. Till now numerous attempts have been made to convert the water soluble Cr(VI), Mn(VII), Ce(IV), Ru(VII), W(VI), Mo(VI) salts to lipopathic with the help of phase transferring quaternary ammonium salts. With appropriate alkyl groups in the quaternary ammonium groups, the salts assemble in both aqueous and nonaqueous media to form organized assemblies mimicking bioaggregates like protein, lipids, and nucleic acids.

In this context, use of amphipathic cetyltrimethylammonium (CTA) ion as the counterion has opened up a new vista to the ongoing oxidizing system. It is well known for its characteristics of being solubilized in both aqueous and nonaqueous media. Unlike other quaternary ammonium ions (tetrabutyl or octyl ammonium ions), CTA has a relatively small head group with more exposed charge and a well-balanced hydrophobic group to carry the ion to both water and organic media and thus is a magic amphiphile. CTA with its counter ion forms tight ion pair in organic solvents, whereas in aqueous medium it dissociates. CTA ion has a balanced amphiphilic system, capable of forming various organized assemblies like micelle in aqueous medium, reversed micelles in organic solvents, microemulsion in aquo-organic medium and even hemimicelles on solid matrix. Surfactant assemblies are of great interest to the organic chemists because of their unusual catalysis of organic reactions and to biochemists because of their similarities to biological membranes and globular proteins.

Besides, in the development of new pharmaceuticals, one crucial task is to elucidate the metabolic fate of the drug candidate in the human body and need to be studied at the earliest stages. Cell-based metabolism studies of active compounds using enzymes liver microsomes, hepatocytes, or microfungi present several advantages but the difficulty of isolation from the biological medium and small quantities of the metabolites are severe drawbacks. Thus in order to perform a satisfactory simulation of enzymatic oxidative metabolism in vivo, several transition metal based non-enzymatic chemical models have been proposed as biomimetic alternative approach to the cell-based metabolism study of bioactive compounds.[220-222]

Some advantages of using in vitro oxidizing system are

- Available in adequate amount for the synthesis of certain metabolites in sufficient amounts with sufficient purity to permit isolation, characterization and further studies.
- The candidate metabolites as available in large quantities can be used to identify the real *in vivo* metabolites and provide sample for pharmacological testing.
- Useful for investigation of metabolic reactions of different substrates and study of their mechanism in different environments.
- The mode of metabolism can be clarified and unstable metabolites can be isolated, detected or trapped under selected and controlled reaction conditions.
- These methods are cheaper compared to the enzymatic model.

With an objective to develop new efficient, selective, mild and biomimetic oxidation protocol, the oxidizing ability of some lipopathic oxidants namely cetyltrimethylammonium dichromate (CTADC), cetyltrimethylammonium permanganate (CTAP) and cetyltrimethylammonium cericnitrate (CTACN) has been explored by our research group using cetyltrimethylammonium (CTA) ion as the onium counterion for anionic dichromate, permanganate and cericnitrate. The

preliminary reports on oxidation of various mono- and bi-functional organic substrates by CTADC and CTAP show their mildness and chemoselectivity as described in the previous section. These oxidants form reverse micellar aggregate in nonpolar medium and forms mixed aggregated structure in presence of various surfactants due to the presence of the cationic carrier CTA^+. Akin to the biological oxidant (enzyme), it provides suitable active site for reaction and thus mimics biological system.

Owing to the above aspects, the present research work aims at employing CTADC and CTAP to study the oxidative cleavage of the following drugs containing multiple reactive functional groups in surfactant generated biomimetic environment. Special attention has been given to the metabolites formed and mechanism of these conversions.

(i) Acetaminophen, an analgesic and anti-inflammatory drug by CTADC

(ii) Epinephrine, a hormone and neurotransmitter by CTADC

(iii) Isoniazid, an anti-tubercular drug by CTADC

(iv) Carbamazepine , an anti-convulsant and antiepileptic drug by CTAP

(v) Norfloxacin, an anti-bacterial drug by CTAP

To achieve the above objectives, the oxidation product(s) were isolated and characterized. The reaction kinetics was studied using UV-Vis spectrophotometric method in different media with varied polarities and also in micro-heterogeneous systems generated due to presence of surfactants. Suitable mechanisms have been proposed and supplemented by proper evidences such as deuterium kinetic isotope effect, solvent kinetic isotope effect, effect of medium, effect of temperature etc. Details on oxidative cleavage of these drugs by CTADC or CTAP has been discussed and presented in the subsequent chapters.

1.6 REFERENCES

1. Starks, C. M.; Owens, R. M. *J. Am. Chem. Soc.* **1973**, *95*, 3616.
2. Starks, C. M.; Liotta, C. *Phase Transfer Catalysis;* Academic: New York, NY, **1978**; Chapter 8.
3. Weber, W. P.; Gokel, G. W. *Phase Transfer Catalysis in Organic Synthesis*; Springer: New York, NY, **1977,** Chapter 11.
4. Dehmlow, E. V.; Dehmlow, S. S. *Phase Transfer Catalysis*; Chemie: Weinheim, **1980**; p 249–255.
5. Lee, D. G. *Oxidation in Organic Chemistry*, Part D; Trahanovsky, W. S., Ed.; Academic: New York, NY, **1982**; Chapter 2.
6. Ammer, J.; Sailer, C. F.; Riedle, E. ; Mayr, H. *J. Am. Chem. Soc.* **2012**, *134*, 11481.
7. Patel, S.; Mishra B. K. *Tetrahedron* **2007,** *63*, 4367.
8. Dash, S.; Patel, S.; Mishra B. K., *Tetrahedron* **2009,** *65*, 707.
9. Piccialli, V.; *Molecules* **2014,** *19*, 6534.
10. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006**, *71*, 6759.
11. Mukherjee, S.; Chakraborty, M.; Panda, A. K.; Bhattacharya, S.C.; Moulik, S.P. *Colloids Surf. A: Physicochem. Eng. Asp.* **2011**, *388*, 1.
12. Wei, M.; Chen, X.; Wang, H.; Li, H.; Wang, Y.; Wang, X.; Huo, M. *Chemosphere* **2013**, *90*, 318.
13. Zhang, Y.; Lu, H.; Wang, L.; Zhang, Y.; Liu, P.; Hana, H.; Jiang, Z.; Li , C. *J. Mol. Catal. A* **2010**, *332*, 59.
14. Lu, H.; Gao, J.; Jiang, Z.; Jing, F.; Yang, Y.; Wang, G.; Li, C. *J. Catal.* **2006**, 239, 369.
15. Li, C.; Jiang, Z..; Gao, J.; Yang, Y.; Wang, S.; Tian, F.; Sun, F.; Sun, X.; Ying, P.; Han, C. R. *Chem. Eur. J.* **2004**, *10*, 2277.
16. Zhang, S.; Gao, S.; Xi, Z.; Xu, J. *Catal. Commun*. **2007**, *8*, 531.
17. Borde, C.; Nardello, V.; Wattebled, L.; Laschewsky, A.; Aubry, J.-M. *J. Phys. Org. Chem.* **2008**, *21*, 652.
18. Lu, H.; Ren, W.; Liao, W.; Chen, W.; Li, Y.; Suo, Z. *Appl. Catal. B: Environ*. **2013**, *138*, 79.
19. Shi, X.; Wei, J. *Appl. Organometal. Chem.* **2007**, *21*, 172.
20. Murugan, R.; Ramamoorthy, K.; Sundarrajan, S.; Ramakrishna, S. *Tetrahedron* **2011**, *67*, 2998.
21. Schmidt, A.K. C.; Stark, C. B. W. *Org. Lett.* **2011**, *13*, 4164.
22. Zhang, Y.; Flowers, R.A. *J. Org. Chem.*, **2003**, *68*, 4560.
23. Jose, N.; Sengupta, S.;. Basu, J.K. *J. Mol. Catal. A* **2009**, *309*, 153.

24. Ghammamy, S.; Eimanieh, H.; Mohammady, M. K. *Synth. Commun.* **2007**, *37*,599.

25. Egoshi, Y.; Kondo, R.; Yoshimoto, Y.; Sugiyama, T.; Usuki, T. *Tetrahedron Lett*. **2013**, *54*, 7029.

26. Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *16*, 2647.

27. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.

28. Hussain, H.; Green, I. R.; Ahmed I. *Chem. Rev.*, **2013**, *113*, 3329.

29. (a) Mangiameli, M. F.; González, J. C.; Bellú, S.; Bertoni, F.; Sala, L. F. *Dalton Trans*., **2014**, *43*, 9242. (b) González, J. C.; Mangiameli, M. F.; Asis, M. C.; Bellú, S.; Sala, L. F. *Polyhedron*, **2013**, *49*, 84. (c) Rizzotto, M.; Levina, A.; Santoro, M.; García, S.; Frascaroli, M. I.; Signorella, S.; Sala, L. F.; Lay, P. A. *J. Chem. Soc., Dalton Trans.* **2002**, 3206. (d) Roldán, V.; González, J.C.; Santoro, M.; García, S.; Casado, N.; Olivera, S.; Boggio, J. C.; Salas-Peregrin, J. M.; Signorella, S.; Sala, L. F. *Can. J. Chem*., **2002**, *80*, 1676. (e) Signorella, S.; Lafarga, R.; Daier, V.; Sala, L. F. *Carbohydr. Res.*, **2000**, *324*, 127. (f) Bellú, S.E. ; González, J.C.; García, S.I.; Signorella, S.; Sala, L.F. *J. Phys. Org. Chem*., **2008**, *21*, 1059. (g) Mangiameli, M.F.; González, J. C.; García, S.; Bellú, S.; Santoro, M.; Caffaratti, E.; Frascaroli, M. J.; Salas Peregrín, J. M.; Atria, A. M.; Sala, L. F. *J. Phys. Org. Chem*., **2010**, *23*, 960.

30. (a)Nakamura, A.; Nakada, M. *Synthesis*, **2013**, *45*, 1421. (b) Weidmann, V.; Maison, W. *Synthesis*, **2013**, *45*, 2201. (c) Sperry, J. *Synthesis*, **2011**, *2011*, 3569. (d) Aïssa, C. *Synthesis*, **2011**, *2011*, 3389. (e) Zhang, Z.; Zhu, G.; Tong, X.; Wang, F.; Xie, X.; Wang J.; Jiang, F. *Curr. Org. Chem.*, **2006**, *22*, 1457.

31. (a) Adcock, J. L.; Barnett, N. W.; Barrow, C. J.; Francis, P. S. *Anal. Chim. Acta*. **2014**, *807*, 9. (b) Stolle, A. *Eur. J. Org. Chem*. **2013**, *2013*, 2265. (c) Khatti, Z.; Davari, M. D.; Bahrami, H.; Zahedi, M. *Prog. React.Kinet. Mec*., **2010**, *35*, 1. (d) Jalce, G.; Franck, X.; Figadère, B. *Tetrahedron: Asymmetry*, **2009**, *20*, 2537. (e) Piccialli, V. Synthesis, **2007**, *2007*, 2585.

32. Soldatenkov, A. T.; Temesgen, A. V.; Kolyadina, N. M. *Chem. Heterocycl. Com*. **2004**, *40*, 537. (b) Salvador, J. A.R.; Silvestre, S. M.; Moreira, V. M. *Curr. Org. Chem*. **2006**, *10*, 2227. (c) Seregar, V. C., Hiremath, C. V., Nandibewoor, S. T. *Trans. Metal. Chem*. **2006**, *31*, 541.

33. Poos, G. I.; Arth, G. E.; Beyler, R. E.; Sarett, L. H. *J. Am. Chem. Soc*. **1953**, *75*, 422.

34. Bernstein, S.; Lenhard, R.H. *J. Am. Chem. Soc.* **1960**, *82*, 3680.

35. Chawla, R. K.; McGonigal, W. E. *J. Org. Chem.* **1974**, *39*, 3281.

36. Gilbert, J. C.; Smith, K. R. *J. Org. Chem.* **1976**, *41*, 3883.

37. Mcmorris, T. C.; Staake, M. D. *J. Org. Chem.* **2002**, *67*, 7902.

38. Bhattacharjee, M. N.; Chaudhui, M. K.; Dasgupta, H. S.; Roy, N.; Khathing, D.T. *Synthesis*, **1982**, 588.

39. Ordonez, M.; Guerrero-de la Rosa, V.; Alcudia, F.; Liera, J. M. *Tetrahedron*, **2004**, *60*, 871.

40. Corey, E. J.; Boger, D. L. *Tetrahedron Lett.*, **1978**, 2461.

41. Hollemberg, D. H.; Klein, R. S.; Fox, J.J. *J. Carbohydr. Res.*, **1978**, *67*, 491.

42. Dauben, W. G.; Michno, D. M. *J. Org. Chem.*, **1977**, *42*, 682.

43. Wada, E.; Okawara, M.; Nakai, T. *J. Org. Chem.*, **1979**, *44*, 2952.

44. (a) Ren, S-K.; Wang, F.; Dou, H-N.; Fan, C-A.; He, L.; Song, Z-L.; Xia, W-J.; Li, D-R.; Jia, Y. X.; Li, X.; Tu, Y-Q. *Synthesis*, **2001**, 2384.(b) Tu, Y-Q.; Ren, S-K.; Jia, Y. X.; Wang, B-M.; Chan, A. S. C.; Choi, M. C. K. *Tetrahedron Lett.*, **2001**, *42*, 2141.

45. Beihoffer, L. A.; Craven, R. A.; Knight, K. S.; Sisson, C. R.; Waddell, T. G. *Trans. Metal Chem.* **2005,** *30*, 582.

46. (a) Piancatelli, G.; Scettri, A.; D'Auria, M. *Tetrahedron Lett.*, **1977**, 3483. (b) Rollin, P.; Sinay, P. *J. Carbohydr. Res.*, **1981**, *98*, 139. (c) Du, Y.; Chen, Q.; Linhardt, R. J. *J. Org. Chem.* **2006**, *71*, 8446.

47. Phillips, D. J.; Pillinger, K. S.; Li, W.; Taylor, A. E.; Graham, A. E. *Chem. Commun.*, **2006**, 2280.

48. Kim, H. J.; Koo, S. *Org. Biomol. Chem*., **2005**, *3*, 3479.

49. Eynde, J-J. V.; Mayance, A.; Maquestiau, A. *Tetrahedron*, **1992**, *48*, 463.

50. McNab, H.; Montgomery, J.; Parsons, S.; Tredgett, D. G. *Org. Biomol. Chem.*, **2010**, *8*, 4383.

51. Zhao, D.; Beiring, B.; Glorius, F. *Angew. Chem. Int. Ed.* **2013**, *52*, 8454.

52. Reddy, M. S.; Narender M.; Rama Rao K. *Tetrahedron* **2007**, *63*, 11011.

53. Yadav, J. S.; Reddy M. S.; Prasad, A. R. *Tetrahedron Lett.* **2005**, *46*, 2133.

54. Yadav, J. S.; Reddy M. S.; Prasad, A. R. *Tetrahedron Lett.* **2006**, *47*, 4995.

55. Piccialli, V.; Zaccaria, S.; Oliviero, G.; D'Errico, S.; D'Atri, V.; Borbone, N. *Eur. J. Org. Chem.* **2012**, 4293.

56. Hunsen, M. Green Technologies for the Environment, ACS Symposium Series, **2014**, Vol. 1186, Chapter 6, pp 117–128.

57. Yu D.; Mattern, D. L.; Forman, B. M. *Steroids* **2012**, *77*, 1335.

58. Kharitonov, Y. V.; Shults, E. E.; Shakirov, M. M. *Chem. Natural Comp.*, **2014**, *49*, 1067.

59. Srikrishna, A.; Lakshmi, B. V.; Sudhakar, A. V. S. *Tetrahedron Lett*. **2007**, *48*, 7610.

60. Heinrich, N.; Willis, A.C.; Cade, I.A.; Ho, J.; Coote, M.L.; Banwell, M.G. *Chem. Eur. J.* **2012**, *18*, 13585.

61. Srikanth, G. S. C. ; Krishna, U. M.; Trivedi, G. K.; Cannon, J. F. *Tetrahedron* **2006,** *62*, 11165.

62. Gowrisankar, S.; Kim, S. J.; Kim, J. N. *Tetrahedron Lett*. **2007,** *48*, 289.

63. Praveen, C.; Hemanthkumar, K.; Muralidharan, D.; Perumal, P. T. *Tetrahedron* **2008,** *64*, 2369.

64. Sriram, R.; Sesha Sai Pavan Kumar, C.N.; Raghunandan, N.; Ramesh, V.; Sarangapani, M.; Rao, V.J. *Synth. Commun.* **2012**, *42*, 3419.

65. Choudhuri, M. K.; Dehury, S. K.; Dhar, S. S.; Sinha, U. B. *Synth. Commun.*, **2004**, *34*, 4077.

66. Nonaka, T.; Kanemoto, S.; Oshima, K.; Nozaki, H. *Bull. Chem. Soc. Jpn.*, **1984**, *57*, 2019.

67. Varadarajan, R.; Dhar, R. K. *Indian J. Chem.*, **1986**, *25B*, 746.

68. Varadarajan, R.; Dhar, R. K. *Indian J. Chem.*, **1986**, *25B*, 971.

69. Narayanan, N.; Balasubramanian, T. R. *Indian J. Chem*., **1986**, *25B*, 228.

70. Patwari, S. B.; Baseer, M. A.; Vibhute, Y. B.; Bhusare, S. R. *Tetrahedron Lett*., **2003**, *44*, 4893.

71. Zhang, Z.; Qian, J.; Tian, Q.; Liu, T.; Liu, Q.; Zhang, G. S*ynth. Commun.*, **2014**, *44*, 674.

72. Murugesan, V.; Pandurangan, A. *Indian J. Chem.*, **1992**, *31B*, 377.

73. Chaudhuri, M. C.; Chettri, S. K.; Lyndem, S.; Paul, P. C.; Srinivas, P. *Bull. Chem. Soc. Jpn.*, **1994**, *67*, 1894.

74. Rajkumar, G. A.; Arabindoo, B.; Murugesan, V. *Indian J. Chem.*, **2000**, *39B*, 74.

75. Tajbakhsh, M.; Mohammadpoor, I.; Alimohammadi, S. K. *Indian J. Chem.*, **2003**, *42B*, 2638.

76. Mohammadpoor Baltork, I.; Pouranshirvani, Sh. *Synth. Commun.*, **1996**, *26*, 1.

77. Mohammadpoor-Baltork, I.; Pouranshirvani, S. *Synthesis*, **1997**, 756.

78. Mohammadpoor-Baltork, I.; Memarian, H.R.; Bahrami, K. *Can. J. Chem.*, **2005**, *83*, 115.

79. Mohammadpoor-Baltork, I.; Sadeghi, M.M.M.; Memarian, H.R.; Pairow, R. *J. Chem. Res.(S)*, **2000**, 40.

80. Hosseinzadeh, R.; Tajbakhsh, M.; Niaki, M. Y., *Tetrahedron Lett.*, **2002**, *43*, 9413.

81. Tajbakhsh, M.; Hosseinzadeh, R.; Niaki, M. Y. *J. Chem. Res.(S)*, **2002**, 508.

82. Tajbakhsh, M.; Hosseinzadeh, R.; Sakoori, A. *Tetrahedron Lett*., **2004**, *45*, 1889.

83. Heravi, M. M.; Bakhtiari, K.; Oskooie, H. A. *ARKIVOC* **2007,** *xiii*, 190.

84. Astles, P. C.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1*, **1997,** 845.

85. Wardrop, D. J.; Bowen, E. G. *Chem. Commun*., **2005**, 5106.

86. Kawamoto, A. M.; Wills, M. *J. Chem. Soc*., *Perkin Trans. 1*, **2001**, 1916.

87. Domostoj, M. M.; Irving, Ed.; Scheinmann, F.; Hale**,** K. J. *Org. Lett.*, **2004,** *6*, 2615.

88. Still, W. C.; Galynker, I. *J. Am. Chem. Soc*., **1982**, *104*, 1774.

89. Bentz, E. L.; Goswami, R.; Moloney, M. G.; Westaway**,** S.M. *Org. Biomol. Chem*., **2005**, *3*, 2872.

90. Yoo, D. J.; Kim, E. Y.; Oelgemöller, M.; Shim, S.C. *Photochem. Photobiol. Sci.*, **2004**, *3*, 311.

91. Nakamura, K.; Baker, T. J.; Goodman, M. *Org. Lett*., **2000**, *19*, 2967.

92. Xing, X.; Fichera, A.; Kumar, K. *Org. Lett.*, **2001**, *3*, 1285.

93. Jana, N.; Mahapatra, T.; Nanda, S. *Tetrahedron: Asymmetry*, **2009,** *20*, 2622.

94. Arnone, A.; Merlini, L.; Nasini, G.; de Pava, O. V.; Zunino, F. *J. Chem. Soc*., *Perkin Trans. 1*, **2001**, 610.

95. D'Auria, M.; Mico, A. D.; D'Onofrio, F.; Scettri, A. *Synthesis*, **1985**, 988.

96. Hector, M.; Hartmann, R.W.; Vincent, V. C. O. *Synth.Commun*., **1996**, *26*, 1075.

97. Maki, S.; Ishihara, J.; Nakanishi, K. *J. Ind. Chem. Soc.*, **2000**, *77*, 651.

98. Morzycki, J. W.; Perez-Díaz, J. O. H.; Santillan, R.; Wojtkielewicz, A. *Steroids* **2010**, *75*, 70.

99. Nyangulu, J. M.; Nelson, K. M.; Rose, P. A.; Gai, Y.; Loewen, M.; Lougheed, B.; Quail, J. W.; Cutler A. J.; Abrams, S. R. *Org. Biomol. Chem.*, **2006**, 4, 1400.

100. Muzart, J. *Synthesis*, **1993**, 11.

101. Chirakul, P.; Sigurdsson, S. Th. *Org. Lett.*, **2003**, *5*, 917.

102. Coleman, R. S.; Gurrala, S. R. *Org. Lett*., **2004**, *6*, 4025.

103. Fleming, I.; Terrett, N. K. *J. Chem. Soc., Perkin Trans. 1*, **1998,** 2645.

104. Mahadevegowda S. H.; Khan, F. A.; *Tetrahedron Lett.* **2014**, *55*, 2266.

105. Mahadevegowda S. H.; Khan, F. A.; *Tetrahedron Lett.* **2014**, *55*, 4400.

106. Dubberke, S.; Abbas, M.; Westermann, B. *Beilstein J. Org. Chem.* **2011**, *7*, 421–425.

107. Mal, K.; Sharma, A.; Das, I. *Chem. Eur. J.* **2014**, *20*, 11932.

108. Santaniello, E.; Milani, F.; Casati, R. *Synthesis* **1983**,749.

109. Ghammami, S.; Seyed Sadjadi, S. A. *J. Serb. Chem. Soc.* **2005**, *70*, 1243.

110. Pourali, A. R.; Goli, A. *Bull. Korean Chem. Soc.* **2006**, *27*, 587.

111. Ghammamy, S.; Mehrani, K.; Afrand, H.; Hajighahramani, M. *Afr. J. Pure Appl. Chem.* **2007**, *1*, 008.

112. Pourali, A. R.; Goli, A. *J. Chem. Sci.* **2011**, *123*, 63.

113. (a) Ghammamy, S.; Baghy, M. R. *J. Chem. Crystallogr*. **2008**, *38*, 907; (b) Ghammamy, S.; Baghy, M. R. *J. Coordn. Chem.*, **2008**, *61*, 3225.

114. (a) Singh, J. V.; Kumar, A.; Srivastava, K.; Mishra, K.; Pandey, A.; Agrawal, G. L. *Oxid. Commun.* **2004**, *27*, 849; (b) Dipti; Tomar, A.; Kumar, A. *Oxid. Commun.* **2012**, *35*, 569.

115. (a) Tomar, A.; Kumar, A. *Asian J. Chem***. 2006**, 18, 3073. (b) Tomar, A.; Kumar, A. *J. Indian Chem. Soc***. 2006**, *83*, 1153. (c) Tomar, A.; Kumar, A. *Oxid. Comm*un. **2007**, *30*, 88. (d) Tomar, A.; Kumar, A. *J. Indian Chem. Soc***.**, **2009**, *86*, 1320.

116. Swami, P.; Vadera, K.; Prasadrao, P. T. S. R. K.; Yajurvedi, D.; Mishra, P.; Sharma, P. K. *Int. J. Chem. Kinet.* **2010**, *42*, 50.

117. Vadera, K.; Sharma, D.; Agarwal, S.; Sharma, P. K. *Indian J. Chem.* **2010**, *49*, 302.

118. Pohani, S.; Pancharia, P.; Purohit, P.; Vyas, S.; Sharma, P. K. *Int. J. Chem. Sci.* **2010**, *8*,1365.

119. Sharma, D.; Pancharia, P.; Vadera, K.; Sharma, P. K. *J. Sulf. Chem*. **2011**, *32*, 315.

120. Vadera, K.; Patel, M.; Vyas, S.; Purohit, P.; Pancharia, P.; Sharma, P.K. *Int. J. Chem. Sci.* **2011,** *9*, 1094.

121. Swami, P.; Malani, N.; Agarwal, S.; Sharma, P.K. *Prog. React. Kinet. Mech.* **2010**, *35*, 309.

122. Pancharia, P.; Purohit, T.; Swami, P.; Malani, N.; Kotai, L.; Prakash, O.; Sharma, P.K. *Int. J. Chem. Sci.* **2012**, *10*, 557.

123. (a)Mahjoub, A. R.; Ghammami, S.; Kassaee, M. Z. *Tetrahedron Lett.* **2003**, *44*, 4555; (b) Gharib, F.; Zare, K.; Ghammami, S.; Ebrahimi, R. *Russ. Chem. Bull*. **2005**, *54*, 462. (c) Ghammamy, S.; Dastpeyman, S.; Sadjadi, S. A. S. *Trans. Metal. Chem*. **2006**, *31*,482.

124. Hajipour, A. R.; Ruoho, A. E. *Synth. Commun*. **2003**, *33*, 871.

125. Hajipour, A. R.; Ruohoa, A. E. *J. Chem. Res. Synop.* **2002**, 547.

126. (a) Mansoor, S. S.; Shafi, S. S. *React. Kinet. Mech. Cat.* **2010**, *100*, 21. (b) Ghammamy, S.; Hashemzadeh, A. *Bull. Korean Chem. Soc.* **2004**, *25*, 1277. (c) Ghammamy, S.; Tajbakhsh, M. *J. Sulf. Chem.* **2005**, *26*, 145. (d) Hassanijoshaghani, A.; Ghammamy, S.; Bagi, S.; Moghimi, A.; Javanshir, Z. *Phosphorus, Sulfur Silicon Relat. Elem.* **2009**, *184*, 164. (e) Ghammamy, S.; Mazareey, M. *Russ. J. Org. Chem.* **2005**, *41*, 1752. (f) Sadjadi, S. A. S.; Ghammagy, S. *Indian J. Chem.* **2006**, *45B*, 564.

127. Zhang, G. S.; Shi, Q. Z.; Chen. M. F.; Cai, K. *Org. Prep. Proced. Intern.* **1998**, *30*, 215.

128. Zhang, G. S.; Yang, D. H.; Chen, M. F. *Synth. Commun*. **1998**, *28*, 3721.

129. Zhang, G. S.; Chai, B. *Synth. Commun.* **2000**, *30*, 1849.

130. Kassaee, M. Z.; Alangi, S. Z. S..; Ghotbabadi, H. S. *Molecules* **2004**, *9*, 825.

131. Alangi, S. Z. S.; Baei, M. T. *J. Mex. Chem. Soc.* **2010**, *54*, 223.

132. Zhang, G. S.; Chai, B. *Synth. Commun.* **2000**, *30*, 2507.

133. Landini, D.; Rolla, F. *Chem. Ind*. **1979**, 213.

134. Murugan, R.; Reddy, B.S.R. *Chem. Lett.* **2004**, *33*, 1038.

135. Suhana, H.; Srinivasan, P. C. *Synth. Commun.* **2003**, *33*, 3097.

136. Chandrasekhar, S.; Takhi, M.; Mohapatra, S. *Synth. Commun*. **1996**, *26*, 3947.

137. Patel, S.; Kuanar, M.; Nayak, B. B.; Banichul, H.; Mishra, B. K. *Synth. Commun*. **2005**, *35*, 1033.

138. Mishra, B. K.; Sahu, S.; Padhan, S.; Patel, S. *Indian J. Chem.* **2009**, *48A*, 1527.

139. Patel, S.; Mishra, B. K. *Tetrahedron Lett.* **2004**, *45*, 1371.

140. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006**, *71*, 3522.

141. Sahu, S.; Patel, S.; Mishra, B. K. *Synth. Commun*. **2005**, *35*, 3123.

142. Sahu, S.; Patel, S.; Mishra, B. K. *Int. J. Chem. Kinet.* **2011**, *43*, 482.

143. Sahu, S.; Sahoo, P. R.; Patel, S.; Mishra, B. K. *Synth. Commun.* **2010**, *40*, 3268.

144. Sahoo, P. R.; Sahu, S.; Patel, S.; Mishra, B. K. *Indian J. Chem.* **2010**, *49A*, 1483.

145. Patel, S.; Mishra, B. K. *Int. J. Chem. Kinet*. **2006**, *38*, 651.

146. Sahoo, P. R.; Patel, S.; Mishra, B. K. *Int. J. Chem. Kinet*. **2013**, *45*, 236.

147. Hajipour, A. R.; Mallakpour, S.E.; Backnejad, H. *Synth. Commun.* **2000**, *30*, 3855.

148. Hajipour, A. R.; Ruoho, A. E. *Sulfur Lett.* **2003**, *26*, 83.

149. Hajipour, A. R.; Mallakpour, S.E.; Malakoutikhah, M. *Indian J. Chem*. **2003**, *42B*, 195.

150. Mohammadpoor-Baltork, I.; Sadhegi, M. M.; Mahmoodi, N.; Kharamesh, B. *Indian J. Chem*., **1997**, *36B*, 438.

151. Mohammadpoor-Baltork, I.; Memarian, H. R.; Hajipour, A. R.; Bahrami, K. *Bull. Korean Chem. Soc.*, **2003**, *24*, 1002.

152. Mohammadpoor-Baltork, I.; Memarian, H. R.; Bahrami, K. *Phosphorous, Sulfur Silicon Relat. Elem.* **2004**, *179*, 2315.

153. Mohammadpoor-Baltork,I.; Memarian, H.R.; Bahrami, K.; Esmayilpour, K. *Phosphorus, Sulfur Silicon Relat. Elem.* **2005**, *180*, 2751.

154. Dilsha, K.M.; Kothari, S. *J. Chem. Sci*. **2009**, *121*, 189.

155. Dilsha, K.M.; Kothari, S. *Oxid. Commun.* **2009**, *32*, 874.

156. Panday, D.; Dilsha, K. M., Kothari, S. *J. Indian Chem. Soc.* **2009**, *86*, 816.

157. Pandey, D.; Kothari, S. *Indian J. Chem.* **2011**, *50B*, 918.

158. Memarian, H.R.; Mohammadpoor-Baltork, I.; Javahery, M. *J. Chinese Chem. Soc.* **2006**, *53*, 511.

159. Hajipour, A. R.; Safaei, S.; Ruoho, A. E. *Synth. Commun.* **2009**, *39*, 3687.

160. Song, Y. H. *Synth. Commun*. **2006**, *36*, 631.

161. Stewart, R. In *Oxidation in Organic Chemistry*; Wiberg, K. B., Ed.; Academic:New York, NY and London, **1965**.

162. Stewart, R. *Oxidation Mechanisms*; Benjamin: New York, NY, **1964**; Chapter 5.

163. Fatiadi, A. J. *Synthesis* **1987**, 85.

164. Carrington, A.; Symons, M. C. R. *Chem. Rev*. **1963**, *63*, 443.

165. (a) Waters, W. A. Q. *Rev. (London)* **1958**, 277; (b) Trost, B. M. *Comprehensive Organic Synthesis (Oxidation)*; Ed.; Pergamon: New York, NY, **1991**; Vol. 7; (c) Stewart, R.; Spitzer, U. A. *Can. J. Chem.* **1978**, *56*, 1273.

166. Kuzhiumparambil, U.; Watanabe S.; Fu, S. *New J. Chem.* **2015**, *39*, 1597.

167. Veprek-Siska, J.; Ettel, V.; Regner, A. *J. Inorg. Nucl. Chem*. **1964**, *26*, 1476.

168. Cullis, C. F.; Ladbury, J. W. *J. Chem. Soc*. **1955**, 555.

169. Gardner, K. A.; Mayer, J. M. *Science* **1995**, *269*, 1849.

170. Wiberg, K. B.; Stewart, R. *J. Am. Chem. Soc*. **1955**, *77*, 1786.

171. Gibson, N. A.; Hosking, J. W. *Aust. J. Chem.* **1965**, *18*, 123.

172. Sam, D.; Simmons, H. E. *J. Am. Chem. Soc.* **1972**, *94*, 4024.

173. Herriott, A. W.; Picker, D. *Tetrahedron Lett.* **1974**, 1511.

174. Sala, T.; Sergent, M. V. *J. Chem. Soc., Chem. Commun.* **1978**, 253.

175. Karaman, H.; Barton, R. J.; Robertson, B. E.; Lee, D. G. *J. Org. Chem*. **1984**, *49*, 4509.

176. Rathore, R.; Chandrasekharan, S. *J. Chem. Res.(S)* **1986**, 458.

177. Holba, V.; Kosicka, R. *Collect. Czech. Chem. Commum*. **1997**, *62*, 849.

178. Mishra, B. K.; Dash, S. *Int. J. Chem. Kinet.* **1995**, *27*, 627.

179. Bank, S.P; Guru, P. S.; Dash, S. *Spectrochim. Acta A.* **2015**, *142*, 34.

180. Bank, S.P; Guru, P. S.; Dash, S. *Carbohydr. Polym.* **2014**, *111*, 806.

181. Brandstrom, A. *Adv. Phys. Org. Chem.* **1977**, *15*, 267.

182. Lee, D. G.; Brown, K. C.; Karaman, H. *Can. J. Chem.* **1986**, *64*, 1054.

183. Hughes, S. R.; Price, D. H. *J. Chem. Soc. A* **1967**, 1093.

184. Krumgal'Z, B. S. *Russ. J. Phys. Chem.* **1971**, *45*, 1448.

185. Gordon, J. E.; Subbarao, G. N. *J. Am. Chem. Soc*. **1978**, *100*, 7445.

186. Weber, W. P.; Shepherd, J. P. *Tetrahedron Lett*. **1972**, 4907.

187. (a) Ogino, T.; Mochizuki, K. *Chem. Lett.* **1979**, 443; (b) Ogino, T. *Tetrahedron Lett*. **1980**, 177.

188. Lee, D. G.; Brown, K. C. *J. Am. Chem. Soc*. **1982**, *104*, 5076.

189. Lee, D. G.; Perez-Benito, J. F. *Can. J. Chem*. **1985**, *63*, 1275.

190. Lee, D. G.; Perez-Benito, J. F. *J. Org. Chem*. **1987**, *52*, 3239.

191. Freeman, F.; Kappos, J. C. *J. Am. Chem. Soc*. **1985**, *107*, 6628.

192. Wiberg, K. B.; Geer, R. D. *J. Am. Chem. Soc.* **1966**, *88*, 5827.

193. Mishra, B. K.; Dash, S. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 673.

194. Mishra, B. K.; Dash, S. *Indian J. Chem.* **1997**, *36(A)*, 662.

195. Mishra, B. K.; Dash, S. *Indian J. Chem*. **2001**, *40(A)*, 159.

196. Brimble, M. A.; Nairn, M. R. *Molecules* **1996**, *1*, 3.

197. Caycho, J. R.; Garcia-Tellado, F.; Armas, P.; Marrero- Tellado, J. J. *Chem. Lett.* **1998**, 25.

198. Schmidt, H. J.; Schafer, H. J. *Angew. Chem., Int. Ed. Engl*. **1979**, *18*, 68.

199. Leddy, B. P.; KcKervey, M. A.; McSweeneey, P. *Tetrahedron Lett.* **1980**, *21*, 2261.

200. Gardner, K. A.; Kuehnert, L. L.; Mayer, J. M. *Inorg. Chem*. **1997**, *36*, 2069.

201. (a) Cook, G. K.; Mayer, J. M. *J. Am. Chem. Soc.* **1994**, *116*, 1855; (b) Cook, G. K.; Mayer, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 7139.

202. Deng, X.; Stefanick, S.; Pippel, M. C. W.; Mani, N. S. *Org. Process Res. Dev*. **2006**, *10*, 1287.

203. Wang, Q.; Wei, Y.; Yue, C. *Speciality Petrochem.*, **2007**, *24*, 26.

204. Srinivasan, N.; Ramadas, K. *Tetrahedron Letters* **2001**, *42*, 343.

205. Maryanoff, C. A.; Stanzion, R. C.; Plampin, J. N.; Mills, J. E. *J. Org. Chem*. **1986**, *51*, 1882.

206. Jain, N.; Xu, J.; Chen, N.; Sui Z. *Tetrahedron Lett*. **2006**, *47*, 225.

207. Paquette, L. A.; Owen, R. D.; Bibart, R. T.; Seekamp, C. K.; Kahane, A. L.; Lanter, J. C.; Corral, M. A. *J. Org. Chem*. **2001**, *66*, 2828.

208. Makosza, M.; Kamienska-Trela, K.; Paszewski M.; Bechcick, M. *Tetrahedron* **2005**, *61*, 11952.

209. Verbeeck, S.; Herrebout, W. A.; Gulevskaya, A.V.; van der Veken, B. J.; Maes, B. U. W. *J. Org. Chem*. **2010**, 75, 5126.

210. Li, C.; Gao, J.B.; Jiang, Z.X.; Wang, S.G.; Lü, Y H.Y.;. Yang, X.; Jing, F. *Topics Catal.* **2005**, *35*, 169.

211. Huang, D.; Lu, Y.C.; Wang, Y.J.; Luo, G.S. *Ind. Eng. Chem. Res*. **2007**, *46*, 6221.

212. Huang, D.; Zhai, Z.; Lu, Y.C.; Yang, L.M.; Luo, G.S. *Ind. Eng. Chem. Res*. **2007**, *46*, 1447.

213. Huang, D.; Wang, Y.J.; Yang, L.M.; Luo, G.S. *Ind. Eng. Chem. Res*. **2006**, *45*, 1880.

214. Zhang, S.; Zhao, G.; Gao, S.; Xi, Z.; Xu, J. *J. Mol. Catal. A* **2008**, *289*, 22.

215. Pai, Z. P.; Kochubey, D. I.; Berdnikova, P. V.; Kanazhevskiy, V. V.; Prikhod'ko, I. Y.; Chesalov, Y. A. *J. Mol. Catal. A* **2010**, *332*, 122.

216. Ma, B.; Zhang, Z.; Song, W.; Xue, X.; Yu, Y.; Zhao, Z.; Ding, Y. *J. Mol. Catal. A: Chem.* **2013,** *368– 369*, 152.

217. Aubry, C.; Chottard, G.; Platzer, N.; Brégeault, J.-M.; Thouvenot, R.; Chauveau, F.; Huet, C.; Ledon, H. *Inorg. Chem.* **1991**, 30, 4409.

218. Duncan, D.C.; Chambers, R.C.; Hecht, E.; Hill, C.L. *J. Am. Chem. Soc*. **1995**, *117*, 681.

219. Adam, W.; Alsters, P.L.; Neumann, R.; Saha-Möller, C.R.; Sloboda-Rozner, D.; Zhang, R. *J. Org. Chem*. **2003**, *68*,1721.

220. Lohman, W.; Karst, U. *Anal. Bioanal. Chem.* **2008**, *391*, 79.

221. Balogh, G. T.; keseru, G. M. *ARKIVOC*, **2004**, *vii*, 124.

222. Akagah, B.; Lormier, A. T.; Fournet, A.; Figadere, B. *Org. Biomol. Chem.* **2008**, *6*, 4494.

Chapter 2

Oxidation of Acetaminophen by Cetyltrimethylammonium Dichromate

2.1 INTRODUCTION

Acetaminophen or paracetamol, is a well-known drug that finds extensive application for its potent analgesic and antipyretic action. It is generally regarded as a non-steroidal anti-inflammatory drug (NSAID), though it possesses little anti-inflammatory activity and also lacks the other typical actions of NSAIDs, such as anti-platelet activity and gastrotoxicity.[1, 2] In spite of acetaminophen's wide use and popularity, its mechanism of action at a molecular level has not yet been completely elucidated. Andersson *et al.*[3] found that the electrophilic metabolites *N*-acetyl-*p*-benzoquinone imine (NAPQI) and *p*-benzoquinone, but not acetaminophen itself, activate mouse and human TRPA1, as a consequence, reduce voltage-gated calcium and sodium currents in primary sensory neurons responsible for decrease in pain and fever.

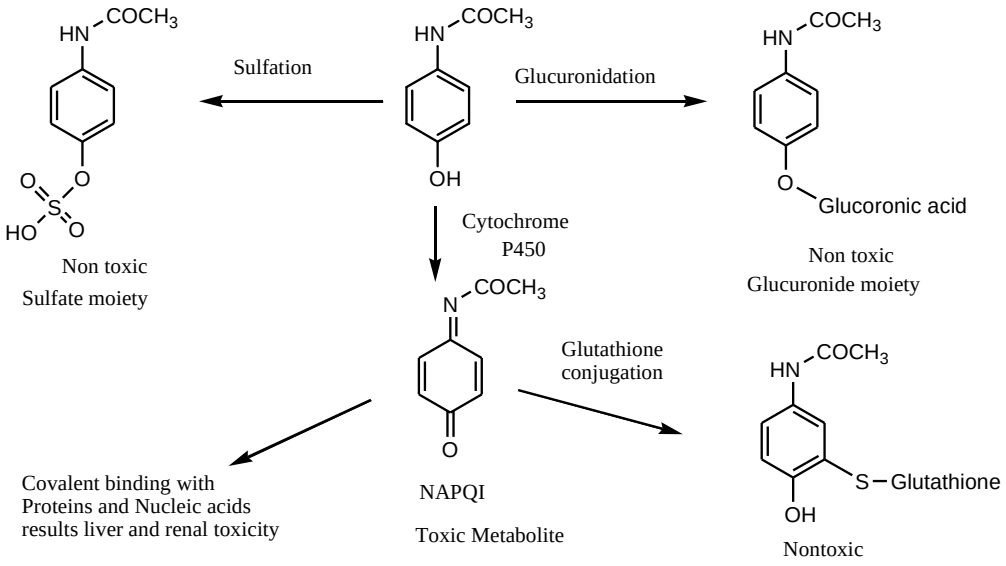
Acetaminophen is metabolized predominantly in the liver into nontoxic products primarily through three metabolic pathways (Scheme 2.1).[4,5] Glucuronidation and sulfation account for more than 80% of the metabolism of the drug to form glucuronide and sulphate conjugates respectively.[4,5] A minor fraction (less than 15%) of the drug is oxidized by cytochrome P450[6] to form *N*-acetyl-*p*-benzoquinone imine (NAPQI)[7,8] and 3-hydroxy-acetaminophen (3-OH-APAP).[9] These oxidized reactive metabolites are inactivated with reduced glutathione to form cysteine and mercapturic acid conjugates. Thus, all three metabolic pathways yield final products that are inactive and non-toxic and are eventually excreted by the kidneys. In the third pathway, however, the intermediate oxidized product NAPQI is toxic. Overdoses of acetaminophen cause liver and renal toxicity as a result of depletion of glutathione and of covalent binding of the excess reactive metabolite to vital cell constituents.[10]

Objectives: In the present section, an attempt have been made to investigate the mechanism of the oxidative metabolism of acetaminophen by CTADC in nonpolar

medium and biomimetic medium generated due to the presence of surfactants to test the following hypothesis.

- Will CTADC act as a mild, chemoselective and biomimetic oxidant for acetaminophen to produce products like NAPQI?
- What will be the mechanism of these conversions? Will it be similar to enzyme catalysed process?
- What will be the effect of different parameters such as reaction temperature, solvent polarity/hydrophobicity, presence of surfactants etc. on the overall rate and mechanism of the oxidative metabolism?

To achieve the above objectives, the oxidation products were isolated and characterized. Kinetics was run in organic media with varied polarities and in microheterogeneous systems generated in presence of various surfactants. The resultant data were analyzed using an appropriate kinetic model. By varying [substrate], [acid], [CTADC] and from the effect of temperature on reaction rate, a suitable mechanism for the reaction was proposed. The solvent kinetic isotope effect and deuterium kinetic isotope effect were also determined to have better insight into the reaction mechanism.



(Scheme 2.1)

Further, kinetic and spectrophotometric determinations of acetaminophen in pharmaceutical formulations has been the subject of interest to many investigators due to their sensitivity, selectivity, simplicity and accuracy.[11] In these methods, the drug (acetaminophen) is oxidized using the metal ion oxidant, and thus, the amount of drug has been estimated. But in most of the methods high concentration of strong acid, high temperature and longer reaction time is required for the process.[12] However, as discussed in Chapter 1, for oxidation of various organic substrates using CTADC does not require any acid in most of the cases, while in some cases it requires a very low concentration of acid (around 5-10% of acetic acid) as compared to conventional Cr(VI) oxidants. Owing to the selectivity, sensitivity, accuracy of kinetic methods and due to the mildness of the oxidant, the oxidation kinetics of acetaminophen by CTADC can be a better alternative to the available methods for the determination of acetaminophen in drug formulations.

2.2 EXPERIMENTAL

2.2.1 Materials

Acetaminophen was purchased from Sigma Aldrich, India and used without further purification. The organic solvents were purified by standard methods.[13] The surfactants cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulphate (SDS) were purified by recrystallization from aqueous ethanol, and their purity was checked from physical constants. Triton X-100 (TX-100) was purchased from Merck, India and was used without further purification. Acetic acid (Merck) was used as source of hydrogen ions and was also used as such. The D_2O (Sigma-Aldrich) used is of NMR grade with 99.8% D.

2.2.2 Synthesis of cetyltrimethylammonium dichromate (CTADC)

CTADC was prepared by following the reported procedure.[14] Aqueous solution of potassium dichromate (2.94 g, 0.05 M) was added slowly to an aqueous solution of CTAB (7.38 g, 0.1 M) with continuous stirring using a teflon coated magnetic bar at room temperature. A yellow coloured compound appeared

immediately. Stirring was continued for 15 minutes more after completion of the addition of the dichromate solution. The resulting yellow product was then filtered off and washed with water several times till no bromide and dichromate were detected in the filtrate. It was vacuum dried and kept in desiccators in the dark. M.P. 212 ^{0}C (decomposed), Yield: 98%

2.2.3 Synthesis of deuterated acetaminophen

Acetaminophen (500 mg) was added to 2 ml of D_2O. To this mixture, acetonitrile was added drop wise until a clear solution was obtained. It was stirred for 1 hour and was kept at room temperature for 3-4 h. The white crystalline solid appeared was isolated by filtration. Comparing the ^{1}H NMR spectrum of D_2O treated acetaminophen (Figure 2.1) with the ^{1}H NMR spectrum of acetaminophen (Figure 2.2), about 70% conversion of N-H to N-D was confirmed.

2.2.4 Kinetic measurements

The oxidation kinetics of acetaminophen by CTADC in acetonitrile in the presence of acetic acid was investigated using UV-vis spectroscopic method by monitoring the decrease in the optical density (OD) of CTADC at an analytical wavelength of 350 nm in a Shimadzu UV-vis spectrophotometer (UV-1800) fitted with thermostatic cell holders. The temperature in the reaction cell was controlled by circulating water by using Lauda thermostat within a temperature fluctuation of 0.05 ^{o}C. The reactions were performed under pseudo-first-order conditions by keeping a large excess (10 times or more) of the acetaminophen with respect to CTADC, and the observed rate constant (k_{obs}) were calculated from the linear plot ($r = 0.99$) of log [CTADC] versus time for up to 75% completion of the reaction.[15,16] The effects of variation of [CTADC], [acetaminophen], [acid] and [surfactant] on the rate constant were investigated by varying the concentration of the desired constituent in the reaction mixture. All experiments were repeated at least three times, and the rates of reactions were obtained with an error of ±4%. Some of the kinetics were run under nitrogen atmosphere, and the data were found to be similar (within an error of ± 2%) to those in normal atmospheric conditions. Hence, most of the reactions were carried

out without nitrogen environment. The solvent isotope effect was investigated using 3:7 mixtures of H_2O/acetic acid and D_2O/acetic acid separately instead of acetonitrile-acetic acid during kinetic studies. Acetic acid was equilibrated with water and D_2O separately for 3 h before its use in the reaction process.

2.2.5 Product analysis

The reaction mixture containing acetaminophen and CTADC in 3:1 ratio in acetonitrile medium was stirred and refluxed for 3 h in the presence of acetic acid. The mixture turned to reddish-green indicating the formation of reduced Cr (III). The completion of the reaction was monitored using TLC. Then the product (M. Pt. =115 ^{0}C) was separated by preparative TLC, taking chloroform and methanol as the solvent, and characterized by IR, NMR spectral analysis. IR Spectrum was recorded on KBr pellets on Perkin Elmer Spectrophotometer.^{1}H and ^{13}C NMR spectra were recorded in a Bruker 400 MHz NMR spectrometer. IR (KBr, ν_{max}/cm^{-1}): An intense peak at 1642 cm^{-1} due to (C=O) stretching, 1317 (Ar C-H bending). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 6.806 (4H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 136.54 and 187.18. IR and NMR spectral data characterized the product to be *p*-benzoquinone. The presence of acetamide in the product mixture was verified from spot test and also comparing with the standard sample.

2.2.6 Stoichiometry

The stoichiometry of the reaction was determined by performing the experiment at 298 K under the condition [CTADC] ≈ [Acetaminophen] at varying acetaminophen concentrations. The disappearance of Cr(VI) was followed until the absorbance values became constant. The [CTADC] after 48 h was estimated. A stoichiometry ratio of [CTADC]/ [Acetaminophen] ≈ 0.35 was observed, which confirmed a 1:3 CTADC: acetaminophen relationship.

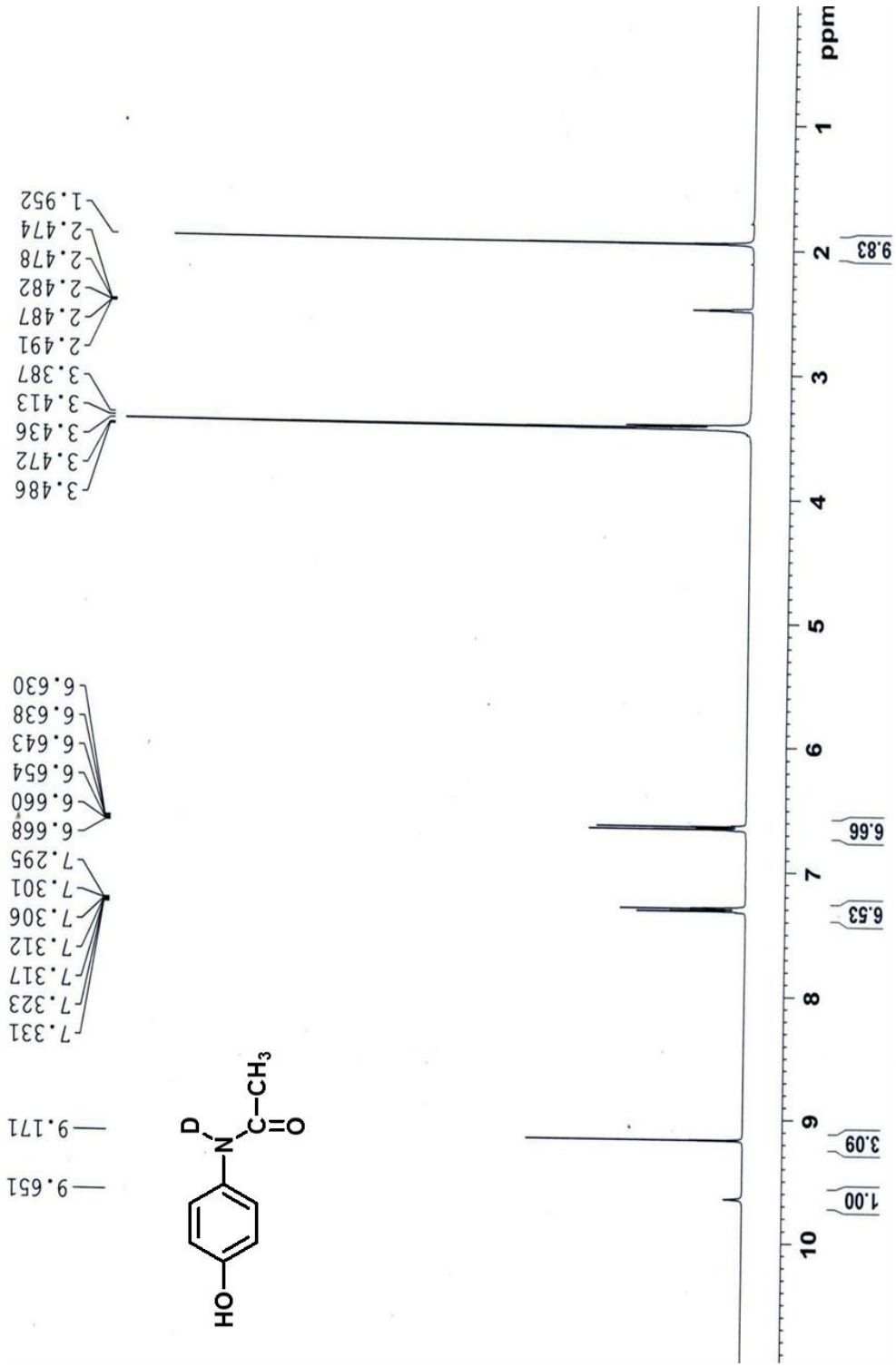


Figure 2.1 ^{1}H-NMR spectrum of deuterated acetaminophen.

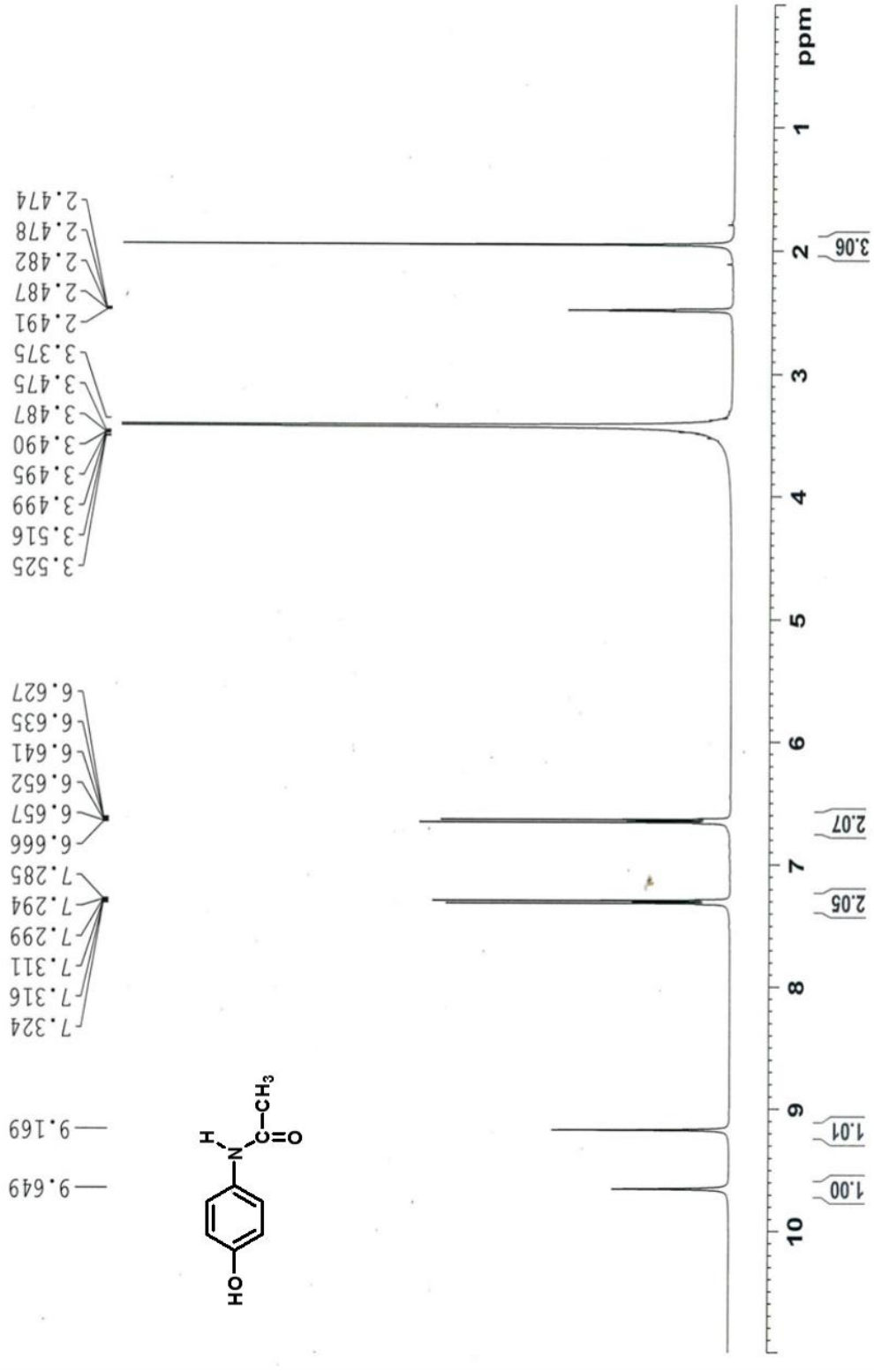


Figure 2.2 ^{1}H-NMR spectrum of acetaminophen.

2.3 RESULTS AND DISCUSSION

Oxidative metabolism of acetaminophen by different oxidizing system is a subject of interest for many investigators to determine the amount of drug in pharmaceutical formulations or for degradation of anthropogenic compounds for treatment of waste and drinking water. Hiremath *et al.*[17] oxidized acetaminophen to quinineoxime by diperiodatoargentate(III) in alkaline medium. Kissinger *et al.*[18,19] and Sanchez-Obrero *et al.*[20] have investigated the electrochemical oxidation of acetaminophen using different electrodes and proved that the first reaction step is an electrochemical oxidation involving two electrons and two protons to generate N-acetyl-p-benzoquinone imine, which further decomposes to stable products by subsequent nonelectrochemical steps. Tabassum *et al.*[21, 22] have proposed free radical pathways for oxidative degradation of acetaminophen to benzoquinone by acidified dichromate and neutral permanganate.

Oxidation of acetaminophen by CTADC in 3:1 molar ratio in acetonitrile-acetic acid mixture afforded *p*-benzoquinone and acetamide along with other products. From the stoichiometric analysis, it has been found that 1 mol equivalent of CTADC reacts with 3 mol equiv of acetaminophen, and therefore the ratio of Cr(VI)/acetaminophen is found to be 1:1.5. Cr(VI) is reduced to Cr(IV) during the reaction process, which is further disproportionate to the final reduced state of chromium (i.e. Cr(III)). The existence of Cr(III) in the product mixture was established from the absorption maximum at 580 nm.[23] The proposal of one electron oxidation giving rise to free radicals may be ruled out for the present reaction due the following two factors: (i) oxidation of acetaminophen, in an atmosphere of nitrogen, failed to induce polymerization of acrylonitrile[16] and (ii) the addition of acrylonitrile did not affect the rate of oxidation reaction.[24]

The observed rate constant (k_{obs}) of the reaction was calculated by monitoring the depletion of chromium(VI) peak at 350 nm in acetonitrile in presence of acetic acid (Table 2.1). The rate constant was found to increase exponentially with increase in the [acetic acid] with almost no reaction in the absence of acid. The logarithmic

plot of k_{obs} versus [acetic acid] shows a second-order dependency with respect to acetic acid. The exponential increase in oxidation rate with increase in [acetic acid] indicates the involvement of protonation of dichromate in the oxidation process. The protonation may be a result of exchange of CTA^+ with H^+ in the acetonitrile/acetic acid medium.

With respect to [Acetaminophen], a Michaelis-Menten type curve was obtained with a fractional order dependency (Figure 2.3 A). Further the Lineweaver-Burk double reciprocal plot was found to be a straight line with an intercept in the y-axis (Figure 2.3 B). Accordingly, a mechanism is proposed, in which a complex forms between the oxidant and the substrate prior to the rate determining step, akin to enzyme substrate complex. In the rate determining step, the complex subsequently decomposes to afford the products (scheme 2.2).

$$\text{Acetaminophen + Oxidant} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \text{Complex}$$

$$\text{Complex} \xrightarrow{k_2} \text{Product}$$

(Scheme 2.2)

Applying steady state approximation to Scheme 2.2, the following rate expression (equations 1-3) can be written.[25]

$$Rate = -\frac{d\,[Oxidant]}{dt} = \frac{Kk_2\,[Acetaminophen][Oxidant]}{1 + K[Acetaminophen]} \quad (1)$$

$$-\frac{d\,[Oxidant]}{dt} \times \frac{1}{[Oxidant]} = k_{obs} = \frac{Kk_2\,[Acetaminophen]}{1 + K[Acetaminophen]} \quad (2)$$

$$\frac{1}{k_{obs}} = \frac{1}{k_2 K\,[Acetaminophen]} + \frac{1}{k_2} \quad (3)$$

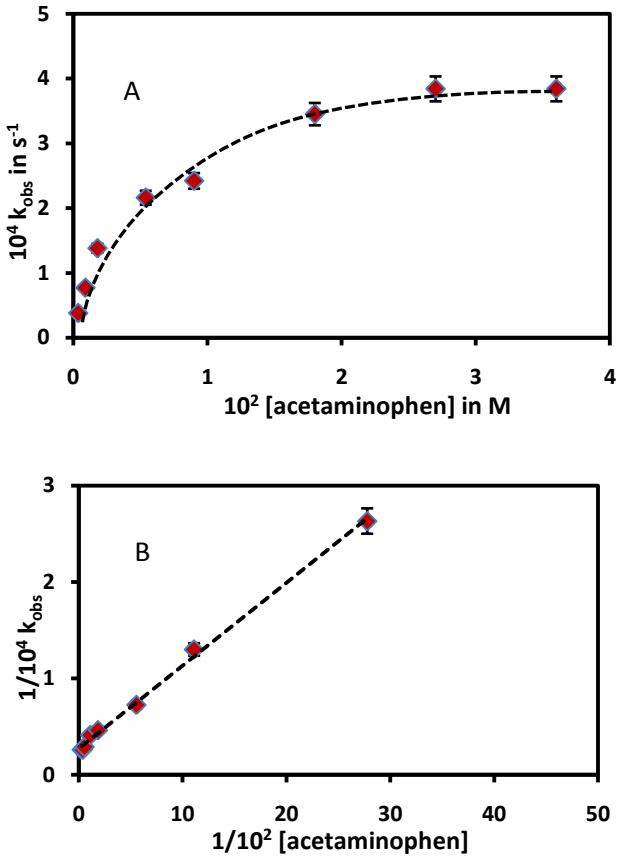


Figure 2.3 (A) Plot of 10^4 x k_{obs} versus 10^2 x [acetaminophen] and (**B**) Plot of $1/(10^4$ x $k_{obs})$ versus $1/(10^2$ x [acetaminophen]) in the oxidation reaction of CTADC with acetaminophen at 298 K.

From the plot of k_{obs} against [acetaminophen] (Figure 2.3 A), the Michaelis-Menten constant, $K_m = ((k_{-1} + k_2)/k_{+1})$ is found to be 0.5 x 10^{-2} M. While investigating the oxidation of acetaminophen by human cytochrome P450 2E1 and 2A6, Chen *et al.* obtained Km values in the range of 0.13 x 10^{-2} to 0.46 x 10^{-2} M.[26] By using the Lineweaver-Burk type double reciprocal equation (equation 3) the equillibrium constant $K = k_{+1}/k_{-1}$ and k_2 are found to be 232 L mol^{-1} and 3.67 x $10^{-4} s^{-1}$ respectively. From K, Km and k_2 values, the k_{+1} and k_{-1} were calculated to be 0.13 and 5.56 x 10^{-4} s^{-1} respectively. The closeness in the k_2 and k_{obs} values also suggests that the decomposition of the complex is the rate-limiting process. The linear plot (R^2 = 0.98) of observed versus predicted rate constant (Figure 2.4) by using k_2 and K values with varying [acetaminophen] supports the kinetic model.

Table 2.1 Effect of [Acetaminophen], [CTADC], and [Acetic acid] on the Oxidation of Acetaminophen by CTADC at 298 K

[CTADC] x 10^4 M	[Acetaminophen] x 10^2 M	[Acetic acid] M	k_{obs} x 10^4 s^{-1}
8.70	0.90	3.18	1.42 (±0.06)
6.54	0.90	3.18	1.50 (±0.06)
4.36	0.90	3.18	1.73 (± 0.07)
2.18	0.90	3.18	2.42(±0.09)
1.30	0.90	3.18	3.22 (±0.14)
0.43	0.90	3.18	3.65 (±0.16)
2.18	3.60	3.18	3.84 (± 0.17)
2.18	2.70	3.18	3.84 (±0.17)
2.18	1.80	3.18	3.45 (±0.15)
2.18	0.54	3.18	2.16 (± 0.08)
2.18	0.18	3.18	1.38 (± 0.06)
2.18	0.09	3.18	0.77(±0.03)
2.18	0.04	3.18	0.38 (±0.02)
2.18	0.90	1.27	0.27 (± 0.01)
2.18	0.90	1.90	0.58 (± 0.03)
2.18	0.90	2.54	1.00 (± 0.04)
2.18	0.90	3.81	3.19 (± 0.14)
2.18	0.90	4.44	3.76 (± 0.15)
2.18	0.90	5.08	4.95 (±0.22)
2.18	0.90	5.71	5.80(± 0.26)
2.18	0.90	6.35	6.83(± 0.29)

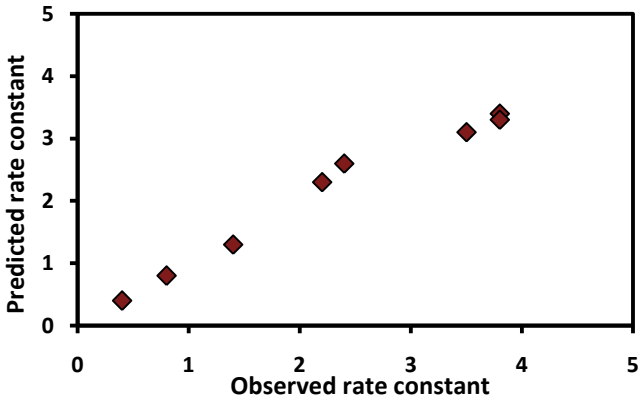


Figure 2.4 Plot of 10^4 x k_{pred} versus 10^4 x k_{obs} in the oxidation of acetaminophen by CTADC in acetonitrile in presence of acetic acid.

With an increase in [CTADC], the rate constant was found to decrease with a fractional order dependency (Figure 2.5). The decrease in the rate constant with increase in [CTADC] is well established in the oxidation of organic substrates by CTADC in organic medium.[27-31] In aqueous media a linear decrease in rate constant with increase in the concentration of Cr(VI) was observed by Menakshisundaram and Vinothini.[32] They explained the fact through the less formation of acid chromate, which is the reactive species in the reaction medium. Similar decreasing trend with increase in [oxidant] has been also observed by other authors.[33] The decrease in rate constant in the present study is attributed to the aggregation of CTADC in organic solvents. Due to the reverse micellar type aggregation of CTADC in organic solvents, the dichromate ion is enveloped by the CTA^+ and the acetaminophen is partitioned into different phases: (i) inner polar core, (ii) cationic reverse micellar interface, and (iii) nonpolar bulk solvent. Therefore, the effective concentration of the substrate in the proximity of the dichromate decreases which leads to a decrease in the rate constant. Further [AcOH] versus the rate constant profile predicts an acid catalyzed reaction. But in the cationic interface of CTADC reversed micelle, a proton may not be available for the dichromate, and therefore a rate decrease is expected. With increasing [CTADC], there may be an increase in aggregation, and thus a negative trend is inevitable.

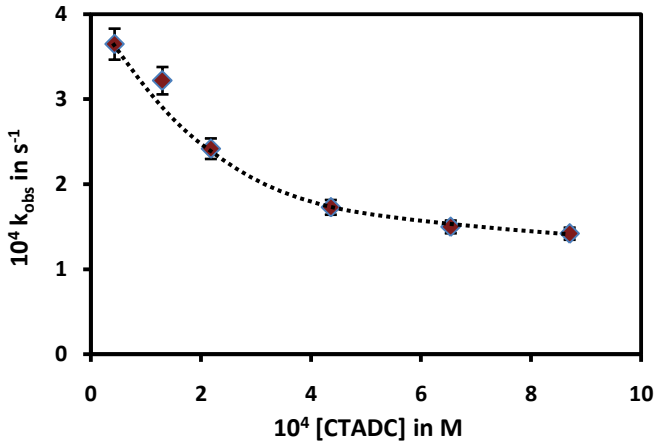


Figure 2.5 Plot of 10^4 x k_{obs} versus 10^4 x [CTADC] in the oxidation reaction of acetaminophen by CTADC in acetonitrile at 298 K.

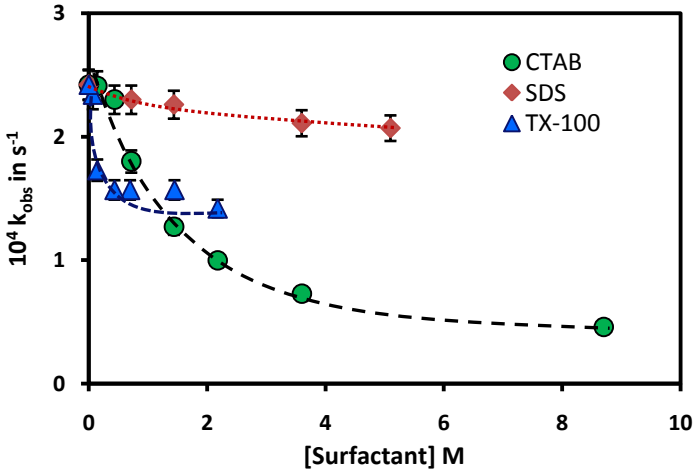


Figure 2.6 Effect of [surfactant] on observed rate constants in the oxidation reaction of acetaminophen by CTADC in acetonitrile in presence of acetic acid at 298 K.

2.3.1 Effect of surfactant

To supplement the above proposition of reverse micellization at higher [CTADC], effect of various surfactants namely CTAB (cationic surfactant), TX-100 (nonionic surfactant), and SDS (anionic surfactant) on the rate of reaction were analyzed. All these surfactants can form reverse micelles in organic solvents,[34-37] mixed aggregated structures (micelles, reverse micelles, microemulsions etc.) with various ionic and nonionicsurfactants,[38-42] and also with CTADC.[30-31] When CTAB was added to the reaction mixture, the rate constant was found to decrease sharply with increasing [CTAB] (Figure 2.6). Above a concentration of 7.2 x 10^{-3} M of

CTAB, the rate constant almost levels up around 0.5 x 10^{-4} s^{-1}. This observation also corroborates the earlier argument of reverse micellar type aggregation and partition of the oxidant and substrate in different subphases. Assuming the rate decrease is only due to the partition effect, for complete entrapment of a dichromate (2.18 x 10^{-4} M) by CTA^+ (7.2 x 10^{-3} M) it requires a composition of 1:33 of CTADC:CTAB. Though to a less extent, addition of nonionic surfactant TX-100 shows similar type of decreasing trend and requires 20 molecules of TX-100 for complete entrapment of 1 molecule of CTADC. If the rate decrease is only due to the partition effect, a substantial decrease in the rate constant with increase in [SDS] is also expected. On the other hand, if the rate change is only due to availability of proton on the reverse micellar interface, micellar catalysis by anionic interface is expected. However, addition of SDS has no remarkable effect on the rate of the reaction. This may be attributed to the neutralization of positive charges of the reverse micellar interface in presence of dodecylsulfate anion of SDS and provide a suitable residing site for H^+, dichromate ion and acetaminophen. Thus the decrease in rate constant due to partitioning of oxidant and substrate may be counter-balanced by the increase in rate due to formation of a suitable environment for possible interaction of oxidant, substrate and acetic acid. These two propositions are described schematically in Figure 2.7. Figure 2.7 A and B represent the repulsion of H^+ from the reverse micellar cationic interface of CTA^+. The extent of repulsion is less in TX-100 reverse micelle, where the positive charge on the interface is only due to CTA^+ from CTADC. Figure 2.7 C shows the interaction between cationic CTA^+ and anionic DS (dodecylsulfate), resulting in charge neutralization on the interface.

From all the above observations, a mechanism is proposed (Scheme 2.3), where CTADC first equilibrates with acetic acid to form a protonated dichromate, which then forms a chromate ester complex with acetaminophen. The complex then decomposes to the products, namely benzoquinone and acetamide.

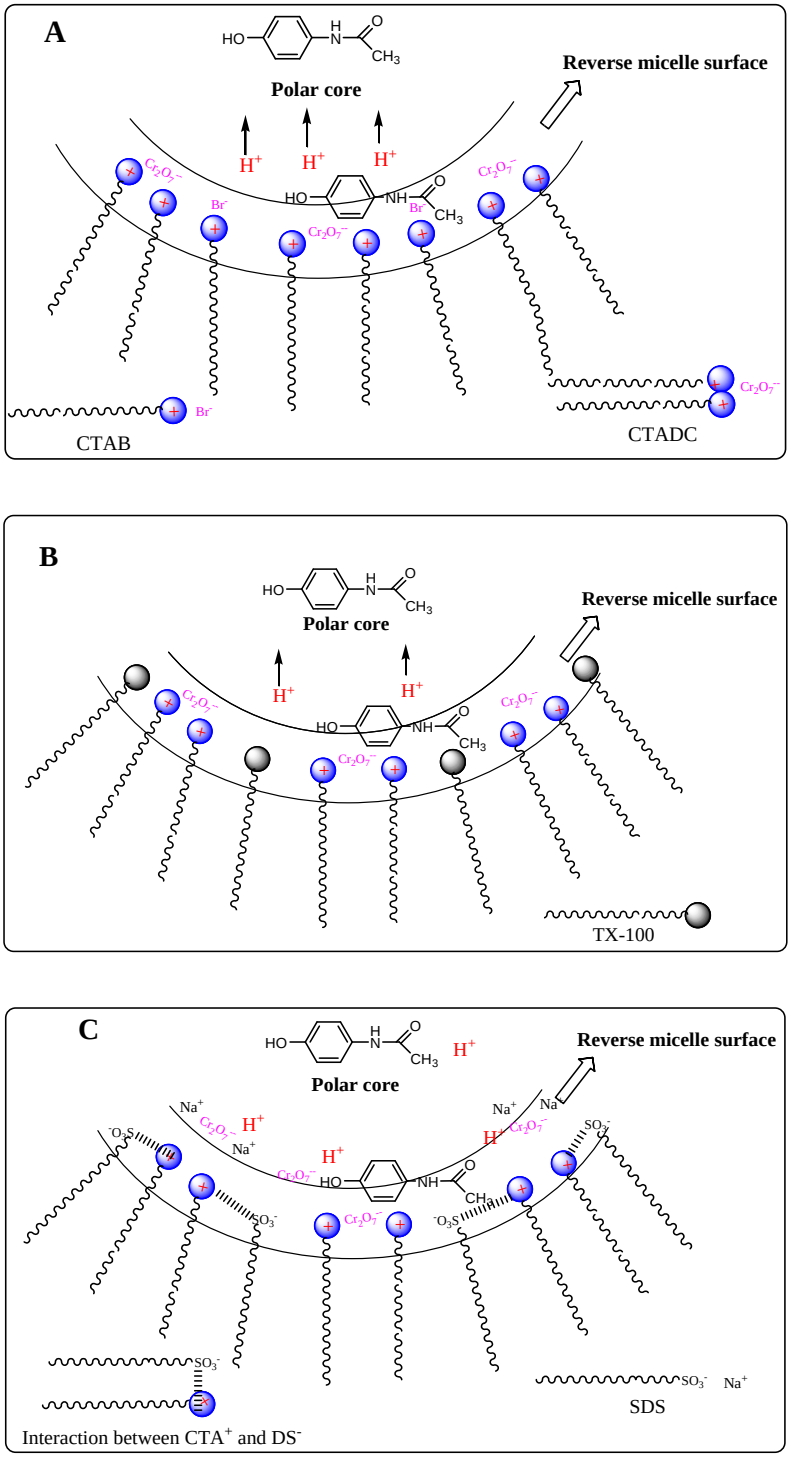
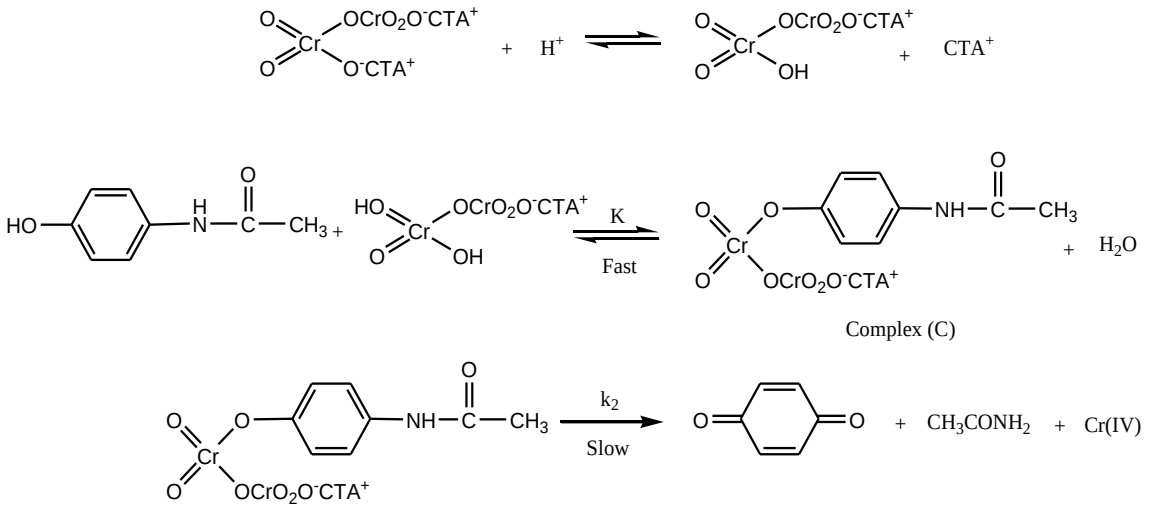


Figure 2.7 Schematic representation of surfactant effect on the oxidation reaction of acetaminophen by CTADC in acetonitrile presence of acetic acid A: Effect of CTAB, B: Effect of TX-100, C: Effect of SDS.



(Scheme 2.2)

2.3.2 Effect of solvent polarity

Investigation of effects of solvent polarity on reactivity always plays a crucial role in determining the nature of the transition state and the mechanism of the reaction. The change in the reaction rates due to change in solvent properties is mainly due to differential solvation of the starting material and transition state by the solvents. When the reactant molecules proceed to the transition state, the solvent molecules orient themselves to stabilize the transition state. If the transition state is stabilized to a greater extent than the starting material, the reaction proceeds faster. If the starting material is stabilized to a greater extent than the transition state, the reaction proceeds slower.

In the present investigation, to observe the effect of environment, the rate of the reaction was measured with change in solvent polarity. The oxidation of acetaminophen by CTADC was studied in solutions containing varying proportions of acetonitrile-acetone and acetonitrile- ethyl acetate in presence of acetic acid. The observed rate constants (Table 2.2 and 2.3) are correlated with different solvent parameters such as $\pi*$ (solvent polarity),[43-44] β (hydrogen bond acceptor basicity),[43-44] *A* (anion solvating power of the solvent),[45] *B* (cation solvating power of the solvent),[45] *A*+ *B* (solvent polarity),[45] log P (logarithm of partition coefficient between octanol and water; referred as solvent hydrophobicity)[46] dielectric constant (ε), dipole moment(μ),

viscosity (η), density (ρ) and surface tension (γ). The polarity parameters for the solvent mixtures have been estimated approximately from the polarity parameters of the pure solvents.

Table 2.2 Observed rate constants in the oxidation of acetaminophen by CTADC at 298 K in acetonitrile-ethylacetate solvent mixture in the presence of acetic acid

Mole fraction (CH_3CN)	Mole fraction (Ethyl-acetate)	$\pi*$	*A*	*B*	β	*Log P*	ε	$10^4 k_{obs(S}{}^{-1})$
0.83	0.00	0.70	0.31	0.71	0.31	-0.28	30.99	2.42(±0.09)
0.82	0.01	0.70	0.31	0.71	0.31	-0.27	30.81	2.42(±0.09)
0.80	0.03	0.69	0.30	0.70	0.31	-0.25	30.05	2.40(±0.09)
0.77	0.05	0.68	0.30	0.69	0.31	-0.23	29.27	2.38(±0.09)
0.71	0.11	0.66	0.28	0.67	0.31	-0.16	27.19	2.29(±0.09)
0.64	0.17	0.63	0.27	0.65	0.31	-0.09	24.90	2.00(±0.08)
0.56	0.24	0.61	0.26	0.62	0.31	-0.02	22.37	1.57(±0.06)
0.47	0.31	0.57	0.24	0.59	0.32	0.07	19.56	1.31(±0.05)
0.37	0.40	0.54	0.22	0.56	0.32	0.16	16.43	0.92(±0.03)
0.26	0.49	0.50	0.20	0.52	0.32	0.27	12.90	0.40(±0.01)

An increase in the polarity and decrease in the hydrophobicity of the medium increased the rate of the reaction. This fact illustrates the formation of a more polar transition state than the reactants in the rate-determining step. Further, the increase in rate constant with an increase in ion solvating power (A + B) of the solvent depicts an ionic transition state for the oxidation reaction. However, similar increasing trend with the increase in both A and B proposes the involvement of both cation and anion in the transition state. On the basis of these observations, it may be proposed that in the rate-determining step, the complex (C) decomposes to the products through an ionic transition state as represented in Scheme 2.4. The non-ideal decrease in the rate

with decrease in solvent polarity (Figure 2.8-2.9) is ascribed to the preferential solvation of the transition state and the positively charged intermediate by acetonitrile.[47] Due to the presence of nonbonding electrons, acetonitrile acts as a good cation solvator.[48] The positive charge in the zwitterionic transition state is localized on the NH^+ moiety, whereas the negative charge is delocalized through the benzene ring and chromate moiety. Accordingly, the transition state (**1**) might behave more as a cation than anion and could be strongly solvated in acetonitrile through ion-dipole interactions, leading to stabilization of the transition state.[49]

Table 2.3 Observed rate constants in the oxidation of acetaminophen by CTADC at 298 K in acetonitrile: acetone solvent mixture

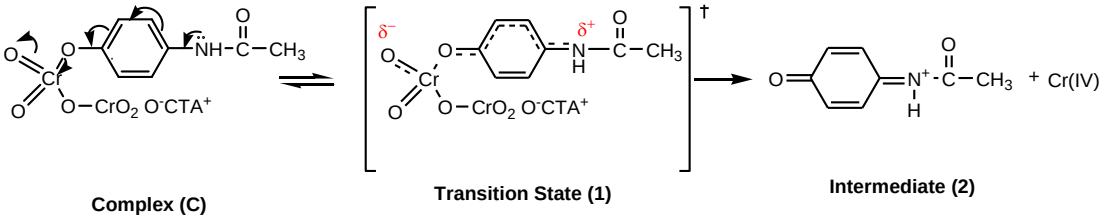
Mole fraction (CH_3CN)	Mole fraction (acetone)	$\pi*$	*A*	*B*	β	*log P*	ε	10^4 x $k_{obs}(s^{-1})$
0.82	0.01	0.71	0.31	0.71	0.31	-0.28	31.05	2.42(±0.09)
0.82	0.01	0.70	0.31	0.71	0.31	-0.28	30.92	2.42(±0.09)
0.79	0.04	0.70	0.30	0.71	0.31	-0.28	30.40	2.42(±0.09)
0.76	0.07	0.69	0.30	0.71	0.31	-0.27	29.87	2.42(±0.09)
0.68	0.14	0.68	0.29	0.70	0.32	-0.27	28.49	2.42(±0.09)
0.60	0.21	0.66	0.28	0.69	0.33	-0.26	27.02	2.05(±0.08)
0.52	0.29	0.65	0.26	0.68	0.33	-0.25	25.48	1.42(±0.06)
0.43	0.38	0.63	0.25	0.67	0.34	-0.24	23.83	0.96(±0.03)
0.33	0.47	0.61	0.24	0.66	0.35	-0.22	22.08	0.65(±0.02)

An analysis of the rate constant values with respect to polarity parameters exhibits some interesting results. From the bilinear plots of k_{obs} against polarity parameters (Figure 2.8-2.9), an ordination between two classes of solvent compositions was experienced. The dissimilar trends in these two groups of solvent

compositions suggest a complex mechanism with varied contribution of these solvents toward the reaction mechanism. The variation of rate constant with change in different parameters (such as concentration of reactants involved, the addition of surfactants and a change in polarity of the medium) shows the importance of two factors toward the mechanism:

(i) Stability of transition state

(ii) Partition of CTADC, acetaminophen and acetic acid in different microheterogeneous phases due to the formation of reverse micelles.



(Scheme 2.4)

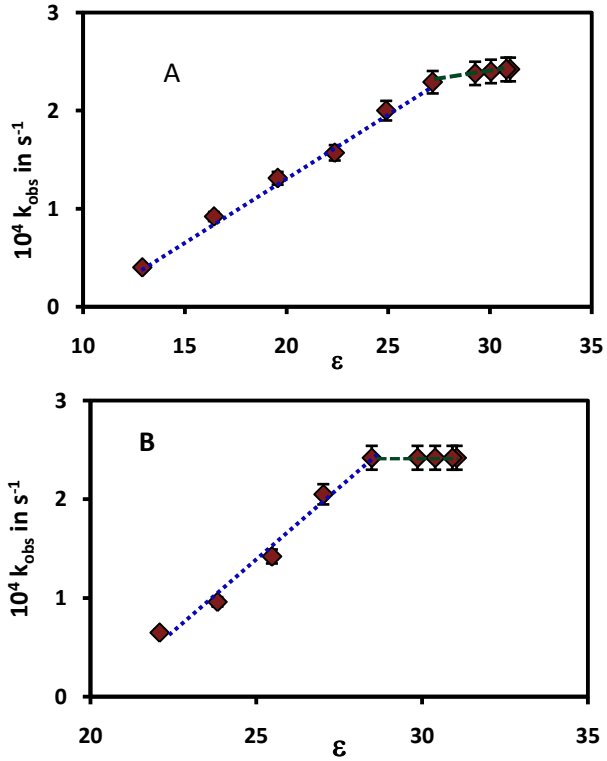


Figure 2.8 Plots of 10^4 x k_{obs} versus dielectric constant of the medium (ε) in the oxidation of acetaminophen in A; acetonitrile-ethyl acetate and B; acetonitrile-acetone binary mixture of solvents. (Dotted lines are drawn manually to show variations)

In the acetonitrile-ethyl acetate mixture (Figure 2.8 A), with the decrease in dielectric constant of the medium from 31 to 27, a slow decrease in the rate constant is observed (slope = 0.02). In the lower side of the plot from dielectric constant values 25 to 10, a sharp decrease is experienced, with slope of 0.13. Thus the decrease in rate constant with decrease in dielectric constant is six times faster in the lower side compared to that of the higher side. If the change in rate constant with change in solvent composition is only due to stability of polar transition state, linear plots of k_{obs} versus polarity parameters are expected.

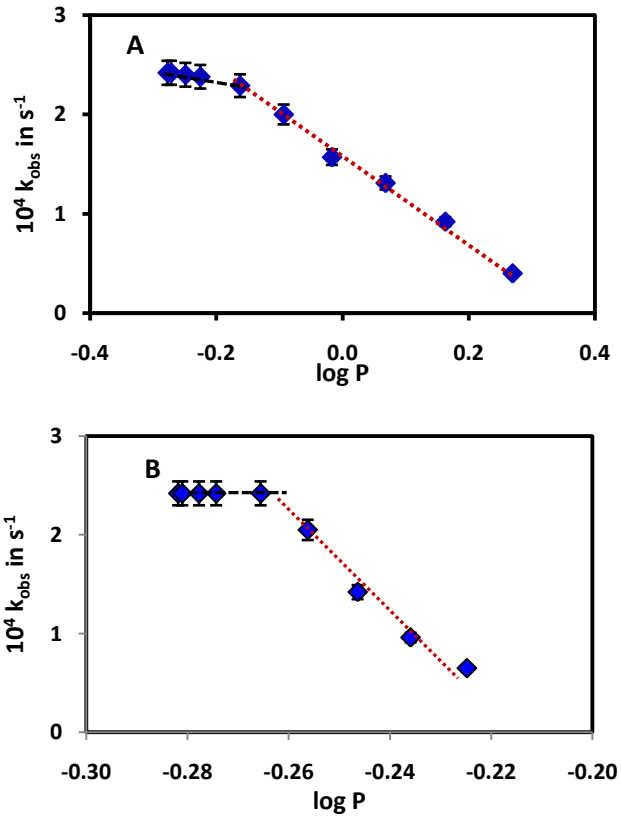


Figure 2.9 Plots of 10^4 x k_{obs} versus log P (solvent hydrophobicity) in the oxidation of acetaminophen in A; acetonitrile-ethyl acetate and B; acetonitrile- acetone binary mixture of solvents. (Dotted lines are drawn manually to show variations)

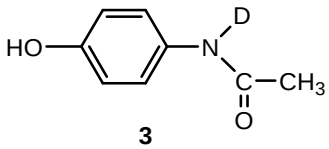
In the mixture of acetonitrile and acetone, similar decrease in rate constant is observed in lower dielectric constant (from 28 to 22) region with slope of 0.29. However, in the higher side, the rate constant remains constant (Figure 2.8 B). The sharp decrease in rate constant in the low polar region is explained through the combining effect of the lesser stability of transition state and the higher ease of

formation of reverse micellar aggregation of CTADC in nonpolar solvents. Quaternary ammonium and phosphonium salts exist as ion pairs or an aggregation of ion pairs in non-aqueous medium.[50] The degree of aggregation of these salts in a nonpolar solvent is inversely proportional to the polarity of the medium.[51] With an increase in nonpolarity of the solvents, reverse micellization increases, which results in a higher partition of oxidant and substrate into two different phases, imparting a rate decrease.

To get further support for the proposed mechanism and transition state, the kinetic isotope effect (k_H/k_D), the solvent kinetic isotope effect $k(H_2O)/k(D_2O)$, and the effect of temperature have been investigated.

2.3.3 Determination of deuterium kinetic isotope effect

Kinetic isotope effect (KIE) has frequently been used to study organic reaction mechanisms and to get information on the structure of the transition state.[52] To study the deuterium kinetic isotope effect, acetaminophen was treated with D_2O to obtain deuterated acetaminophen. By comparing the ^1H NMR spectrum of the D_2O treated acetaminophen (Figure 2.1) with the ^1H NMR spectrum of acetaminophen (Figure 2.2) and also with the literature report, [53] approximately 70% exchange of N-H proton to form deutero acetaminophen (**3**) was suggested. Using 3 as the substrate and keeping all other parameters constant, rate constant for the oxidation of deuterated substrate i.e. k_D was measured. No significant primary kinetic isotope effect (k_H / k_D) was observed. If the rate determining step involves the breaking of a N-H bond, a large deuterium isotope effect is to be expected. Because no isotope effect is found in the present reaction, it is concluded that the rate determining step does not involve the breaking of an N-H bond. Therefore, it may be assumed that the complex(C) decomposes to the N-acetyl-p-benzoquinone iminium ion in the rate-determining step, which on deprotonation furnished NAPQI in a fast process. NAPQI is further hydrolyzed to form benzoquinone and acetamide in the acidic medium (Scheme 2.5).



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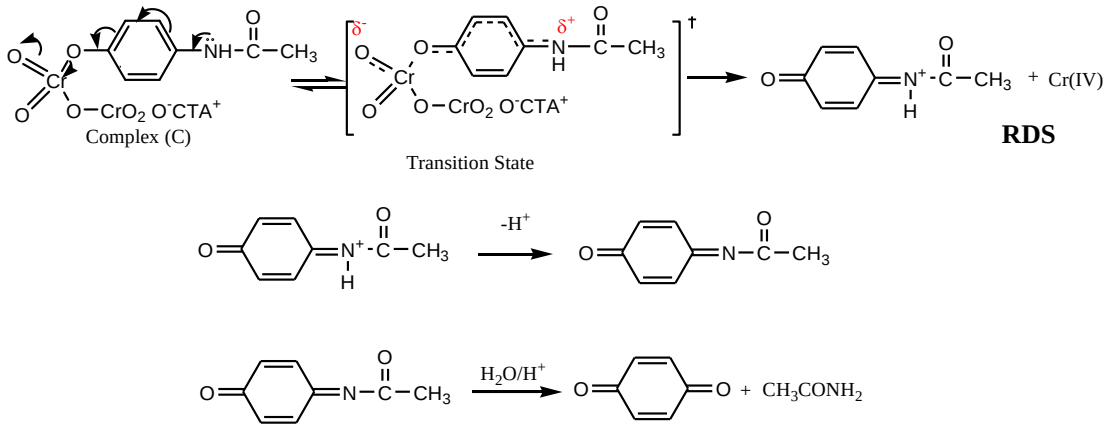
2.3.4 Determination of solvent kinetic isotope effect

Isotopic labelling on solvents may influence the rate of the reaction and sometimes gives useful information for the elucidation of reaction mechanism. To obtain the solvent kinetic isotope effect ($k(H_2O)/k(D_2O)$), the reaction was carried out in 3:7 mixture of H_2O-acetic acid and D_2O-acetic acid separately. The solvent kinetic isotope effect, $k(H_2O) / k(D_2O)$, was found to be $((1.57 \times 10^{-3})/ (1.29 \times 10^{-3}) = 1.22)$. The magnitude of the isotope effect in this case is similar to that of a secondary isotope effect and it may be explained through the differential solute-solvent interaction due to isotopic substitution.[54] On isotopic substitution, the physical characteristics of the solvents change; consequently, the strength of hydrogen bonds also change. The hydrogen bond strength is higher in D_2O compared to water.[55] The hydrogen bonding energy in D_2O is 929 J mol^{-1} more negative than in H_2O at 298.15K.[56,57] Both the reactant and the transition state formed stronger hydrogen bonds with D_2O than water and thus stabilized more, leading to higher drop in free energy in D_2O. However, as proposed in Scheme 2.4 and 2.5, there is charge induction in the transition state; thus, the difference in free energy of the transition states in D_2O and in water is predicted to be less compared to the free energy difference of the reactants in both the solvents (Figure 2.10). Calculating the thermodynamic parameters in both the cases (Table 2.4), this difference is found to be 0.4 (± 0.03) kJ mol^{-1}. Consequently, the solvent kinetic isotope effect is greater than one, though considerably smaller.



(Scheme 2.5)

2.3.5 Effect of temperature

With increase in temperature, the rate constant was found to increase linearly (Table 2.4). The thermodynamic parameters like activation energy (E_a), enthalpy of activation ($\Delta H^{\ddagger}$), entropy of activation ($\Delta S^{\ddagger}$) and free energy of activation ($\Delta G^{\ddagger}$) were calculated using Arrhenius and Eyring equation and found to be 48.7 kJ mol^{-1}, 46.2 kJ mol^{-1}, -160 J mol^{-1} K^{-1} and 93.7 kJ mol^{-1} respectively in acetonitrile. The proposed mechanism is also supported by the values of thermodynamic parameters. The lower energy of activation and high free energy of activation support the formation of highly solvated transition state. The high negative entropy of activation is also indicative of an extensively solvated activated complex than the reactants.[58] The formation of ions from neutral molecules passes through a polar transition state, implying a drop in entropy due to extensive solvation and $\Delta S^{\neq}$ assumes a larger negative value. Further, with a decrease in the solvent polarity, the value of $\Delta S^{\neq}$ became higher negative showing an appreciable increase in ordering in less polar solvents (Table 2.4). All these experiments support the formation of an ionic polar transition state.

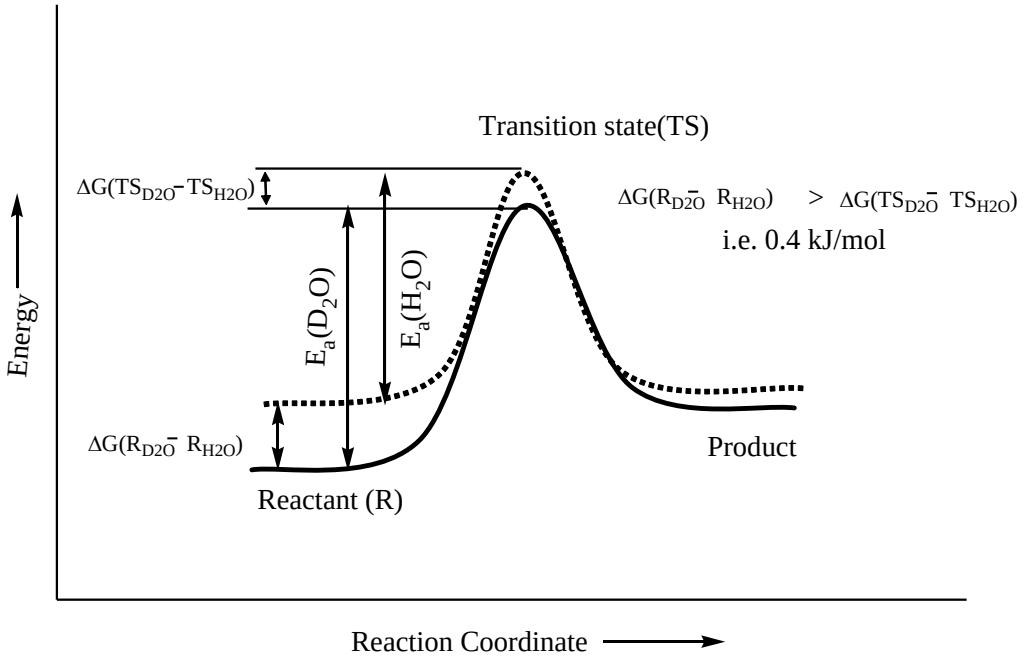


Figure 2.10 Free energy diagram (Solid line: D_2O solvated reactant and TS; dotted line: H_2O solvated reactant and TS) depicts drop in free energy of reactant and transition state due to solvation in D_2O. The difference in free energy of reactants ΔG (R_{D2O}-R_{H2O}) is greater than the difference in free energy of transition states ΔG (TS_{D2O}-TS_{H2O}) due to difference in the strength of hydrogen bonds.

Table 2.4 Activation parameters determined in different solvents for the oxidation of acetaminophen by CTADC.

Solvent	10^4 k_{obs} (s^{-1})					$\Delta H^{\neq}$	$\Delta G^{\neq}$	$\Delta S^{\neq}$
	293K	298K	303K	308K	313K	kJ/mol	kJ/mol	J mol^{-1} K^{-1}
Acetonitrile	1.59 ±0.06	2.42 ±0.09	3.44 ±0.12	4.45 ±0.14	5.77 ±0.24	46.22	93.74	-159.47
Ethylacetate	1.08 ±0.04	1.46 ±0.05	2.03 ±0.08	2.65 ±0.11	-	42.87	94.89	-174.44
Dioxane	0.47 ±0.02	0.64 ±0.02	0.84 ±0.04	1.11 ±0.04	-	40.35	96.97	-189.90
H_2O-Acetic acid(3:7)	10.40 ±0.34	15.74 ±0.53	19.65 ±0.63	27.11 ±1.06	-	44.01	89.14	-151.41
D_2O-Acetic acid(3:7)	9.40 ±0.29	12.86 ±0.38	17.96 ±0.58	23.41 ±0.84	-	43.61	89.49	-153.90

2.4 CONCLUSION

In conclusion, the oxidant (CTADC) oxidizes acetaminophen to *p*-benzoquinone following Michaelis-Menten kinetics. The first step involves a very fast formation of an association complex between acetaminophen and CTADC and is followed by a second rate determining dissociation of the complex proceeding via a polar transition state to form N-acetyl-p-benzoquinone iminium ion intermediate, which on subsequent deprotonation and hydrolysis forms the product. CTADC assembles to form reverse micelles in nonpolar solvents and mixed reverse micelles with other surfactants and provides different residing sides for reactants. The substrate and oxidant are thus partitioned into two different types of environments; accordingly, the reaction is controlled. The outcome from solvent effect illustrates the contribution of reverse micellization and the stability of transition state toward the rate of the reaction. The proposed mechanism also gets support from the solvent isotope effect, the deuterium kinetic isotope effect and the effect of temperature on the reaction rate.

2.5 REFERENCES

1. Chandrasekharan, N. V.; Dai, H.; Roos, K. L. T.; Evanson, N. K.; Tomsik, J.; Elton, T. S.; Simmons, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13926.
2. Botting, R. M. *Clin. Infect. Dis.* **2000**, *31*, 8202.
3. Andersson**,** D. A.; Gentry, C.; Alenmyr, L.; Killander**,** D.; Lewis, S. E.; Andersson**,** A.; Bucher**,** B.; Galzi, J. L.; Sterner, O.; Bevan, S.; Hogestatt, E. D.; Zygmunt**,** P. M. *Nat. Commu.* **2011**, *2*, 551.
4. Forrest, J. A.; Clements, J. A.; Prescott, L. F. *Clin.Pharmacokinet.* **1982**, *7*, 93.
5. Prescott, L. F. *Br. J. Clin. Pharmac.***1980**, *10*, 291S.
6. Dong, H.; Haining, R. L.; Thimmel, K. E.; Rettie, A. E.; Nelson, S. D. *Drug Metab. Dispos.* **2000**, *28*, 1397.
7. Dahlin, D. C.; Miwa, G. T.; Lu, A. Y.; Nelson, S. D. *Proc Natl. Acad. Sci. USA*. **1984**, *81*, 1327.
8. Corcoran, G. B.; Mitchell, J. R.; Vaishnav, Y. N.; Horning, E. C. *Mol. Pharmacol.* **1980**, *18***,** 536.
9. Hinson, J. A.; Pohl, L. R.; Monks, T. J.; Gillette, J. R.; Guengerich, F. P. *Drug Metab. Dispos.***1980**, *8*, 289.
10. Parmar, D.; Kandakar, M. *Eur. J. Pharmacol.* **1995**, *293*, 225.
11. Shrestha, S. R.; Pradhananga, R. R. *J. Nep. Chem. Soc.* **2009**, 24, 39.

12. Sultan, S. M. *Talanta* **1987**, *30*, 605.

13. Riddick, J. A.; *Bunger*, W. B. *Organic Solvents*; Techniques of Chemistry, Vol. II, Wiley-Interscience: New York, 1970.

14. Patel, S.; Mishra, B. K. *Tetrahedron Lett.* **2004**, *45*, 1371.

15. Naik, P. N.; Chimatadar, S. A.; Nandibewoor, S. T. *Ind. Eng. Chem. Res*. **2009**, *48*, 2548.

16. Khan, A. A. P.; Asiri, A. M.; Azum, N.; Rub, M. A.; Khan, A.; Al-Youbi, A. O. *Ind. Eng. Chem. Res.* **2012**, *51*, 4819.

17. Hiremath, D. C.; Hiremath, C. V.; Nandibewoor, S. T. *Eur. J. Chem.* **2006**, 3, 13.

18. Kissinger, P. T.; Roston, D. A.; Benschoten, J. J. V.; Lewis, J. Y.; Heineman, W. R. *J. Chem. Educ*. **1983**, *60*, 772.

19. Miner, D. J.; Rice, J. R.; Riggin, R.M.; Kissinger, P. T. *Anal. Chem.* **1981**, *53*, 2258.

20. Sanchez-Obrero, G.; Mayen, M.;.Mellado, J. M. R.; Rodriguez-Amaro, R. *Int. J.Electrochem. Sci*. **2011**, *6* , 2001.

21. Tabassum, S.; Sabir, S.; Sulaiman, O.; Hashim, R. *J. Dispersion Sci. Technol*. **2011**, *32*, 710.

22. Tabassum, S.; Sabir, S.; Sulaiman, Rafatullah, M.; Khan, I.; Hashim, R. *J. Dispersion Sci. Technol*. **2011**, *32*, 217.

23. Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochmann, M. In *Advanced Inorganic Chemistry*, 6th ed.; John Wiley & Sons: New York, 1999; p 747.

24. Hegde, R. N.; Shetti, N. P.; Nandibewoor, S. T. *Ind. Eng. Chem. Res*. **2009**, *48*, 7025.

25. Laidler, K. J. In *Chemical Kinetics*, 2nd ed.; McGraw-Hill: New York, 1968; p 327.

26. Chen, W.; Koenings, L. L.; Thompson, S. J.; Peter, R. M.; Rettie, A. E.; Trager, W. F.; Nelso, S. D. *Chem. Res. Toxicol.* **1998**, *11*, 295.

27. Sahoo, P. R.; Patel S.; Mishra, B. K. *Int. J. Chem. Kinet.* **2013**, *45*, 236.

28. Sahu, S.; Sahoo, P.; Patel, S.; Mishra, B. K. *Synth. Commun*. **2010**, *40*, 3268.

29. Patel, S.; Sung, D. D.; Mishra, B. K. *Indian J. Chem*. **2008**, *47A*, 1218.

30. Patel, S.; Mishra, B.K. *J. Org. Chem*. **2006,** *71*, 3522.

31. Patel, S.; Mishra, B.K. *J. Org. Chem*. **2006**, *71*, 6759.

32. Meenakshissundaram, S.; Vinithini, R. *Croat. Chem. Acta* **2003**, *76*, 75.

33. Wiberg, K. B. Oxidations in Organic Chemistry; Academic Press, New York, 1965; p 78.

34. Rosso, F. D.; Bartoletti, A.; Profio, D. P.; Germani, R.; Savelli, G.;Blasko, A.; Bunton, C. A. *J. Chem. Soc., Perkin Trans.*, **1995**, *2*, 673.

35. Fletcher, P. D. I.; Galal, M. F.; Robinson, B. H. *J. Chem. Soc. Faraday Trans*. *1*,**1985**, *81*, 2053.

36. Tessy, E. I.; Rakshit, A. K. *Bull. Chem. Soc. Jpn*. **1995**, *68*, 2137.
37. Jimenez-Carmona, M. M.; Luque de Castro, M. D. *Anal. Chem*. **1998**, *70*, 2100.
38. Majhi, P. R.; Blume, A. *J. Phy. Chem. B* **2002**, *106*, 10753.
39. Yue, H.; Guo, P.; Guo, R. *J. Chem. Eng. Data* **2009**, *54*, 2923.
40. Tah, B.; Pal, P.; Mahato, M.; Talapatra, G. B. *J. Phys. Chem. B*, **2011**, *115*, 8493.
41. Chakraborty, H.; Sarkar, M. *Langmuir*, **2004**, *20*, 3551.
42. Cui, X.; Jiang, Y.; Yang, C.; Lu, X.; Chen, H.; Mao, S.; Liu, M.; Yuan, H.; Luo, P.; Du, Y. *J. Phys. Chem. B*, **2010**, *114*, 7808.
43. Kamlet, M. J.; Abboud, J. L. M.; Abraham. M. H.; Taft, R. W. *J. Org. Chem*. **1983**, *48*, 2877.
44. Taft, R. W.; Abboud, J. L. M.; Kamlet, M. J. *J. Org. Chem*. **1984**, *49*, 2001.
45. Swain, C. G.; Swain, M. S.; Powel, A. L.; Alunni, S. *J. Am. Chem. Soc.* **1983**, *105*, 502.
46. Katritzky, A. R.; Fara, D. C.; Kuanar, M.; Hur, E.; Karelson, M. *J. Phys. Chem. A* **2005**, *109*, 10323.
47. Patel, S.; Gorai, S.; Malik, P. K. *J. Photochem. Photobiol.* A **2011**, *219*, 76.
48. Reichardt, C. Solvents and solvent effects in organic chemistry, 22nd ed.; VCH publishers Ltd.: Cambridge, 1988; p 69.
49. Um, I. H.; Kang, J. S.; Park, J. Y. *J. Org. Chem.* **2013**, *78*, 5604.
50. Brandstrom, A. *Adv. Phys. Org. Chem*. **1978**, *15*, 267.
51. Starks, C. M.; Owens, R. M. *J. Am. Chem. Soc*. **1973**, *95*, 3613.
52. Ammal, S. C.; Mishima, M.; Yamataka, H. *J. Org. Chem.* **2003**, *68*, 7772.
53. Santos, M. da S.; Colnago, L. A. *Quimica Nova* **2013**, *36*, 324.
54. Ruff, F.; Csizmadia, I. G.; In Organic reactions Equilibria, kinetics and mechanism, Elsevier: New York, **1994**: Pg. 240.
55. Scheiner, S.; Cuma, M. *J. Am. Chem. Soc.* **1996**, *118*, 1511.
56. Marcus, Y. *Russian J. Electrochem.* **2008**, *44*, 16.
57. Marcus,Y. *Ions in water and biophysical implications*, Springer, New York, 2012; pg17.
58. Khan, A. A. P.; Mohd, A.; Bano, S.; Siddiqi, K. S. *Ind. Eng. Chem. Res.* **2011**, *50*, 9883.

Chapter 3

Oxidation of Epinephrine by Cetyltrimethylammonium Dichromate

3.1 INTRODUCTION

Epinephrine, commonly known as adrenaline, is a hormone and neurotransmitter belonging to the catecholamine family.[1] It liberates glucose into the blood stream through a variety of enzymatic reaction and finally stimulates the body to make a spontaneous decision to fight or flight.[2-4] The role of epinephrine as a neurotransmitter highly depends upon its oxidation mechanism. Inside the body adrenaline is oxidized by an enzyme known as amine oxidase, and in that case adrenaline molecule is oxidized in the side chain.[5] At an intermediate pH (6-8), epinephrine in aqueous buffer is oxidized to red coloured substance known as adrenochrome.[6] Auto-oxidation of epinephrine produces hematin, methemoglobin and adrenochrome.[7-10]

Literature reports a good deal of examples for the oxidation of epinephrine to various coloured organic products and intermediates by different homogeneous and heterogeneous oxidizing systems.[11-23] It is proposed that organic radicals are involved in these oxidations of epinephrine. Fenton's reagent at pH 4.5 oxidizes adrenaline to adrenochrome by a free-radical mechanism.[11] In the catalytic oxidation of epinephrine to adrenochrome by mesoporous silica nanoparticles (MSN) as heterogeneous catalyst, it is reported that large surface area, characteristic mesoporosity and surface structures facilitate the deposit of reactants inside MSN particles, and catalyze the oxidation process.[18] Szigyarto *et al*.[19] have studied the Mn(II) complex catalyzed oxidation of epinephrine to adrenochrome at room temperature. They proposed that the catalytic effect is mainly due to the binding of the catalyst to dioxygen and the substrate and formation of a ternary complex between catalyst-dioxygen-substrate as active intermediate. Lupano *et al*.[20,21] have used hydrogel based Co(II) catalyst complex for H_2O_2 activation for the oxidation of epinephrine to adrenochrome. With the use of a Co(II)-poly(EGDE-DA) complex, about 77% of conversion was achieved in 30 min, while with the use of Co(II)-Poly(EGDE-MAA-2MI) about 80% conversion of epinephrine to

adrenochrome was achieved in less than 6 min, following a pseudo-first-order kinetic model.

Objectives: Owing to the selectivity, mildness and biomimetic characteristics of CTADC the main objective aims to investigate the oxidative metabolism of epinephrine by CTADC. The specific objectives of the present section are to analyze the following hypothesis.

- Can CTADC act as a mild, chemoselective and biomimetic oxidant for epinephrine? What will be the product of the oxidative degradation?
- What will be the mechanism of the conversion? Will it be a free radical process or ionic process?
- What will be the effect of different parameters such as reaction temperature, solvent polarity/hydrophobicity, acidity and presence of surfactants etc. on the overall rate and mechanism of the oxidative metabolism?

To get a clear picture of the oxidative metabolism, mechanism of the oxidative conversion has been investigated through kinetic study in surfactant generated biomimetic medium.

3.2 EXPERIMENTAL

3.2.1 Materials

CTADC was synthesized as per the procedure discussed in Chapter 2. Epinephrine was purchased from Sigma Aldrich, India and used without further purification. Glacial acetic acid (Merck, India) was used without further purification. The surfactants CTAB and SDS were purified by recrystallization from aqueous ethanol, and their purity was checked from physical constants. TX-100 was purchased from Merck, India and was used without further purification. Acetonitrile was purified by standard method.

3.2.2 Kinetic measurements

The oxidation kinetics of epinephrine by CTADC in acetonitrile in the presence of acetic acid was investigated by monitoring the appearance of the product adrenochrome at an analytical wavelength of 455 nm in a Shimadzu UV-vis spectrophotometer (UV-1800) fitted with thermostatic cell holders. The temperature in the reaction cell was controlled by circulating water by using a Lauda thermostat within a temperature fluctuation of 0.05^{o}C. All reactions were carried out under pseudo-first-order conditions, keeping excess of [CTADC] with respect to [epinephrine]. Pseudo-first-order rate constants (k_{obs}) were calculated from the linear plots of ln ($A_\infty - A_t$) vs. t. The effect of variation of [CTADC], [epinephrine], [acid] and [surfactant] on the rate constant was investigated by varying the concentration of the desired constituent in the reaction mixture. All experiments were repeated at least three-times and the rates of reactions were obtained within an error of ±6%.

3.2.3 Calculation of isokinetic temperature

The isokinetic temperature was calculated following Exner's method.[24] In this method, two rate constants (k_1 and k_2) at two different temperatures (T_1 and T_2) are determined for a series of reaction. A linear plot of log k_1 vs. log k_2 confirms the validity of isokinetic relationship and the isokinetic temperature β, is calculated using the following two equations (equation 1 and 2). Taking T_1 = 308 K and T_2 = 303 K, for the present reaction the isokinetic temperature was calculated to be 366K.

$$\log k_2 = b \log k_1 + a \qquad (1)$$

$$\beta = T_1T_2\,(1\text{-}b)/\,(T_1\text{-}T_2b) \qquad (2)$$

3.2.4 Stoichiometry

Limiting logarithmic method was used to determine the Stoichiometry of the oxidation reaction.[25-26] For this, two different sets of experiments were done. In the first set of experiment, the epinephrine concentration was varied (0.6 x 10^{-5} to 0.9 x

10^{-4} M) at a fixed CTADC concentration (5.8 x 10^{-4} M) and in the second set of experiment the CTADC concentration was varied (4.6 x 10^{-4} to 1.4 x 10^{-3} M) at a fixed epinephrine concentration (0.6 x 10^{-4}M). The absorbances of the product were recorded after a fixed time interval (20 minutes) after the mixing of reactants. The logarithms of the obtained absorbances were plotted against log [epinephrine] and log [CTADC] in the first and the second set of experiments respectively. The ratio of the slopes of the fitting lines represents the stoichiometry of the reaction and found to be 3:2 epinephrine to CTADC, respectively.

3.3 RESULTS AND DISCUSSION

Under reflux conditions, the solution of CTADC and epinephrine in acetonitrile yielded a red colored product, which was identified as adrenochrome from the occurrence of a broad absorption peak at 455 nm in the UV-vis absorption spectrum of the reaction mixture (Figure 3.1).[18,19,23,27,28] Extraction of a dirty green residue from the reaction mixture illustrates the reduction of Cr(VI) to Cr(III) in the reaction process. Using limiting logarithmic method stoichiometry of the reaction was calculated to be 3:2 epinephrine to CTADC respectively.[25,26]

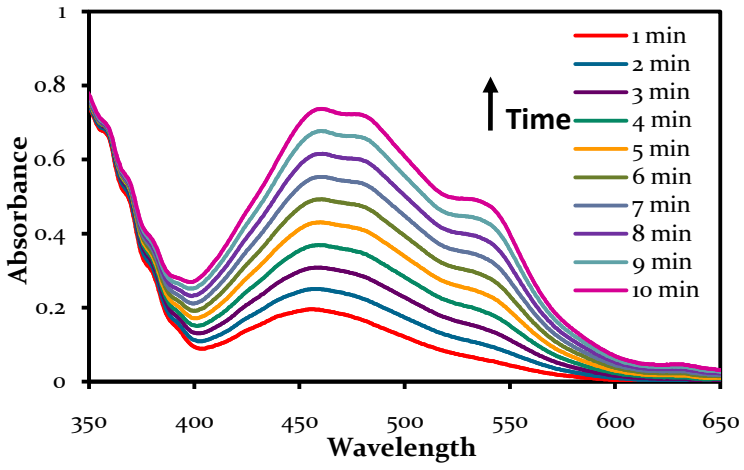


Figure 3.1 UV-vis spectra of reaction mixture for the reaction of CTADC and epinephrine in thepresence of acetic acid in acetonitrile showing the product formation at different time interval.

Reaction kinetics of epinephrine with CTADC in the presence of acetic acid was followed spectrophotometrically by monitoring the appearance of the product adrenochrome at an analytical wavelength of 455 nm (Figure 3.1). Though the reaction is also possible in the absence of acetic acid, epinephrine is sparingly soluble in acetonitrile in the absence of acetic acid. Hence kinetics was studied in presence of a fixed amount of acetic acid in the medium. The rate constants were measured under pseudo-first-order conditions with the concentration of CTADC maintained in more than 10-fold excess relative to the substrate concentration. All reactions obeyed first-order kinetics. Pseudo-first-order rate constants (k_{obs}) were calculated from the linear plots of ln ($A_\infty - A_t$) vs. t. From the k_{obs} values corresponding rates were calculated by multiplication of the k_{obs} values with the [substrate]. The k_{obs} and rate values at different reaction conditions are summarized in Table-3.1.

3.3.1 Effect of reactant concentration

From the linear logarithm plot of rate versus [substrate], the order of reaction with respect to epinephrine was found to be 1.35. Using multiple regression analysis, log (rate) values obtained at different conditions were correlated with the parameters of the reaction condition, that is, [substrate] and [oxidant], to obtain a relationship between the rates of reaction with the parameters of the reaction condition. The regression model, thus obtained, has been presented in equation 3. Accordingly the rate expression can be written as in equation 4. The constant term (i.e. -1.337) in the regression model demonstrates that when the reactant concentration tends to 1 the rate constant and hence the rate tends to 4.6 x 10^{-2} s^{-1}.

Log (rate) = -1.337(±0.186) - 0.138(±0.059) log [CTADC] +1.349(±0.03) log [Epinephrine] (3)

R^2 = 0.996, F = 1071 n = 12

Rate= 0.046 $[CTADC]^{-0.14}$ $[Epinephrine]^{1.35}$ (4)

Table 3.1 Effect of [Epinephrine] and [CTADC] on the Oxidation of epinephrine by CTADC in acetonitrile at 298 K

[CTADC] $x10^4$ (M)	[Epinephrine]x 10^4 M)	$k_{obs} \times 10^4$ (s^{-1})	Rate x 10^8 (M s^{-1})	
			Observed	Calculated[a]
5.8	0.06	17.2 ± 0.9	1.03	1.17
5.8	0.12	25.0 ± 1.2	3.00	2.97
5.8	0.18	31.8 ± 1.6	5.72	5.12
5.8	0.3	37.9 ± 1.9	11.37	10.20
5.8	0.5	42.2 ± 1.9	21.10	20.32
5.8	0.6	43.1 ± 2.1	25.86	25.98
5.8	0.9	44.5 ± 2.2	40.05	44.88
4.65	0.6	44.5 ± 2.1	26.70	26.78
9.31	0.6	39.8 ± 1.9	23.88	24.35
11.64	0.6	38.5 ± 1.9	23.10	23.61
13.96	0.6	38.2 ± 1.8	22.92	23.03

[a]Calculated using Eq (1)

Using the regression model, the log (rate) values were predicted and plotted against the observed values (Figure 3.2). A linear plot without any outlier supports the regression model.

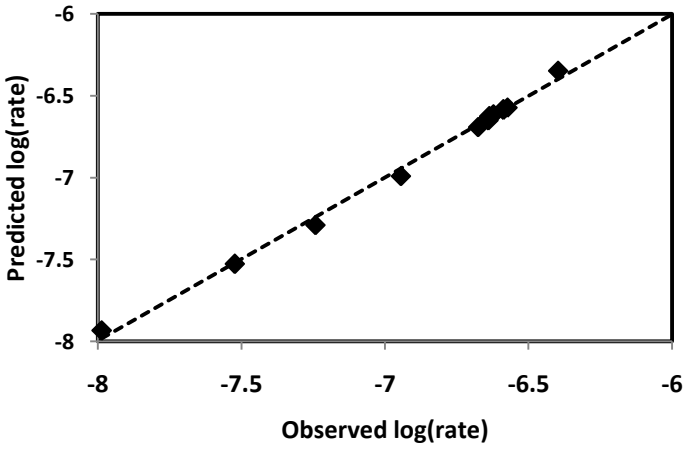
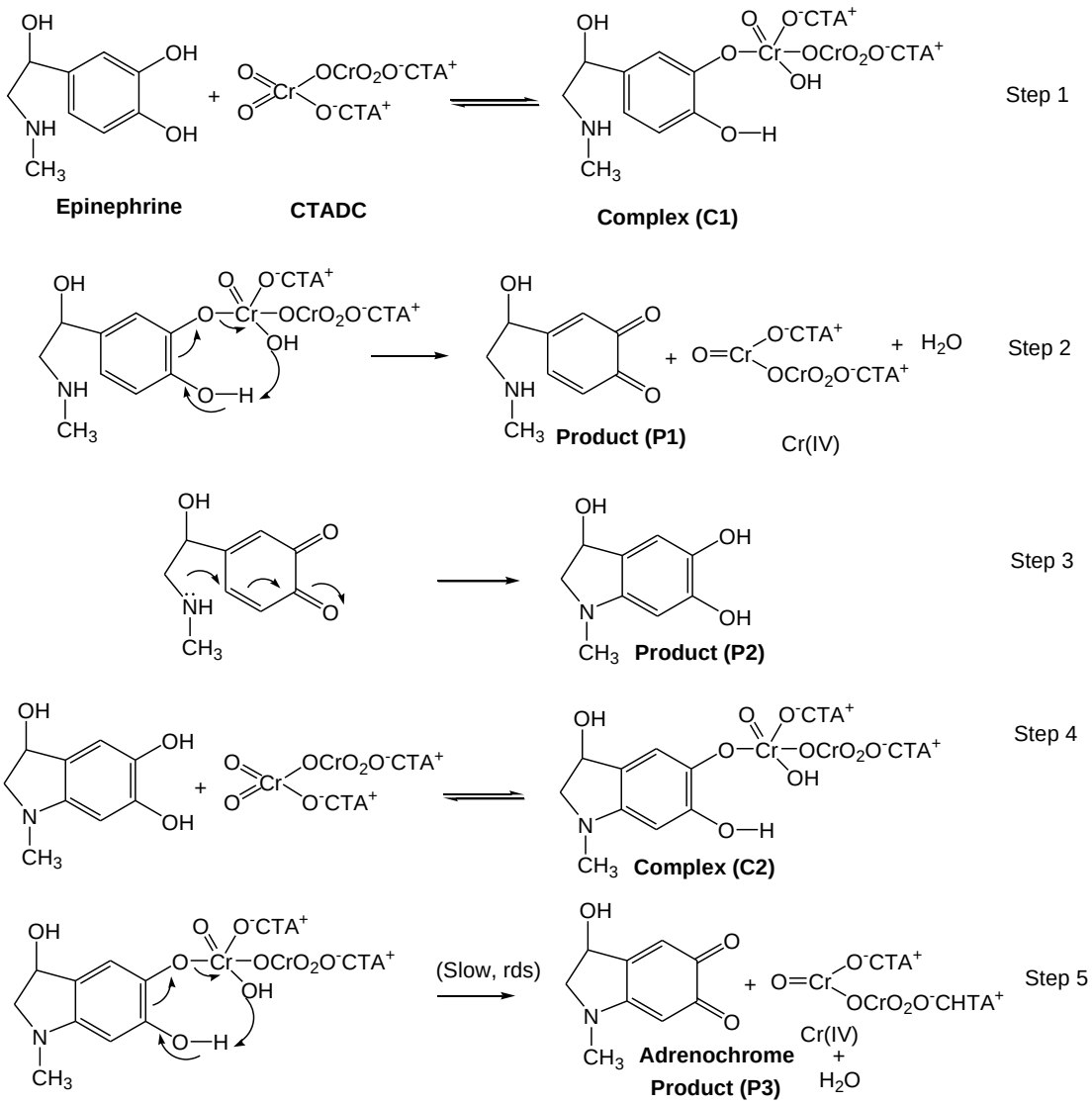


Figure 3.2 Plot of predicted versus observed log (rate) for the oxidation reaction of epinephrine with CTADC in acetonitrile at 298 K.

Oxidation of hydroxylic and phenolic substrates by CTADC goes through a multistep reaction process, where the first step is the formation of a 1:1 association complex between the oxidant and the substrate.[29-33] The complex subsequently decomposes to the products through rate-determining hydrogen abstraction by the chromate oxygen. In the present study the fractional orders with respect to the oxidant and the substrate (equation 4) indicate the occurrence of a complex reaction mechanism, which may be proposed as in Scheme 3.1. Epinephrine first forms a complex C1 with CTADC which then decomposes to form epinephrine quinone (**P1**). Intramolecular cyclization through a nucleophilic attack by the nitrogen on the quinone ring of **P1** followed by aromatization through proton transfer afforded leucochrome (**P2**). As reported earlier, the half-life of the primary oxidation product (**P1**) was only 0.06 sec; that is, this open chain quinone exists only as a very transient intermediate between epinephrine and adrenochrome.[34] In presence of Cr(VI), leucochrome further oxidizes to the product adrenochrome (**P3**) through the rate determining decomposition of complex **C2**.

The proposed mechanism gets support from the rate retarding effect of acetic acid. With an increase in the concentration of acetic acid, the rate of the reaction was found to decrease linearly (equation 5 and Figure 3.3). As per equation 5, in the absence of acetic acid, the rate of the reaction will be 32.67×10^{-8} M s^{-1}. In acidic medium epinephrine exists in the cationic form **(1)** and is oxidized to the cationic quinone **(2).**[34] The protonation at nitrogen prevents the nucleophilic attack for possible intramolecular cyclization to form the **P2**. With a decrease in acidity the increase in formation of free base leads to cyclization, and as a result, the rate of the reaction increases. If, step 2 would have been the rate-determining step, there would not be any rate retardation with increase in [acetic acid], rather a rate increase would have been observed because of an increase in formation of protonated dichromate and fast formation of Complexs **C1** and **C2**. Thus, step-5 may be proposed to be the rate determining step. With an increase in acidity, [P2] decreases, affecting the [C2] and thus affecting the rate of the reaction.



(Scheme 3.1)

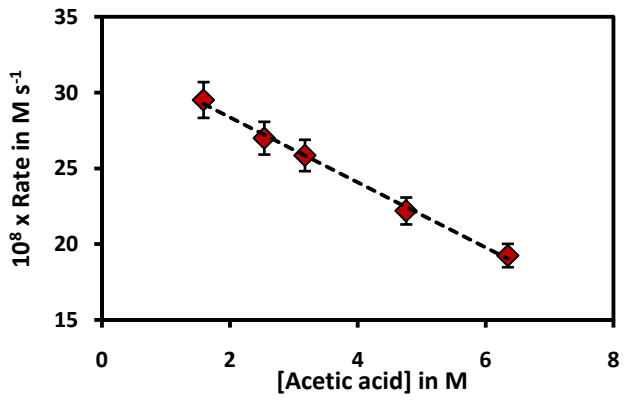
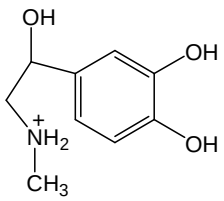
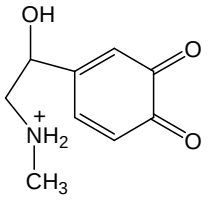


Figure 3.3 Plot of rate of the reaction versus [acetic acid] for the oxidation of epinephrine with CTADC in acetonitrile at 298 K.

Rate = -2.1458 [Acetic acid] + 32.67 $R^2 = 0.997$ (5)



1



2

After the removal of the organic products, the presence of green coloured residue in the reaction mixture supports the reduction of Cr(VI) to Cr(III).[35] Free radical mechanism through one electron transfer may not be possible because of the following: (i) no precipitate was observed by addition of acrylonitrile to the reaction mixture.[36-38] (ii) there is no signal in the EPR spectrum; (iii) the addition of acrylonitrile does not have any effect on the rate of reaction. Thus, during the oxidation process, Cr(VI) was reduced to Cr(IV). The reduced Cr(IV) by a disproportionation reaction in a sequential manner further changed to Cr(III) (Scheme 3.2). The existence of Cr(IV) as the reduced state in oxidation of various organic substrates by different chromium oxidants including onium chromates and dichromates is well established.[39,40]

$$Cr(VI) + Epinephrine \rightarrow Cr(IV) + Adrenochrome$$

$$Cr(IV) + Cr(VI) \rightarrow 2\ Cr(V)$$

$$Cr(V) + Cr(IV) \rightarrow Cr(III) + Cr(VI)$$

(Scheme 3.2)

Similar to the oxidation of acetaminophen by CTADC (Chapter 2), a nonlinear decrease in rate with increase in [CTADC] is observed (Table 3.1) and can be explained through the possible formation of reverse micellar type aggregates. The dichromate ions thus remain enveloped by the CTA^+ leading to decrease in the effective concentration of the substrate in the proximity of the dichromate.

3.3.2 Effect of surfactant

To supplement the above proposition, the effect of surfactants such as CTAB, TX-100 and on the rate of the reaction was analyzed. Asymptotic decreasing profiles in the rates are observed with increase in surfactant concentrations (Figure 3.4). The break points in these plots may provide evidence for the formation of mixed reverse micellar aggregate of CTADC with the added surfactant and partition of reactants in different subphases. In presence of SDS, a significantly higher decrease is observed as compared to the CTAB and TX-100. SDS forms a reverse micelle with negative charge at the interface and thus provides a favorable residing site for H^+. Previous reports on acetic acid catalyzed oxidation of various organic substrates by CTADC, opposite trend has been observed with respect to [SDS].[29-32] The results were explained through the stabilization of protonated dichromate in the anionic interface of SDS reverse micelle. In the present study, the protonated dichromate is also stabilized in the SDS reverse micellar interface (Scheme 3.3), which may increase the concentration of **C1** leading to an enhancement in the formation of **P1**. On the other hand, it may decrease the concentration of **P2** (which is supposed to be the reactant for the second oxidative process), due to stabilization of protonated epinephrine quinone (**2**) resulting in a decrease in the rate.

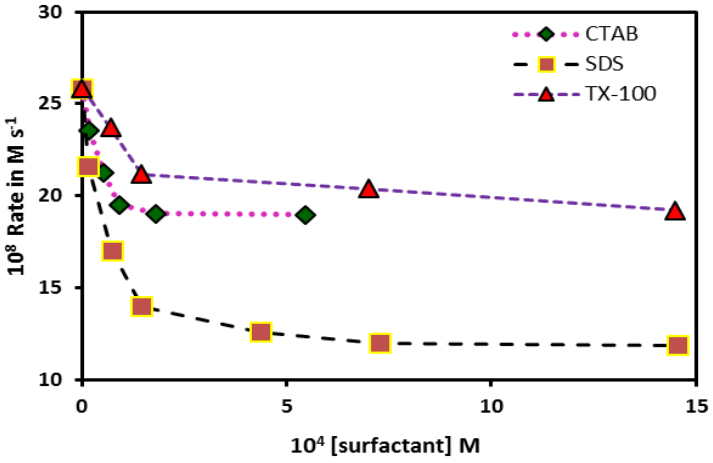
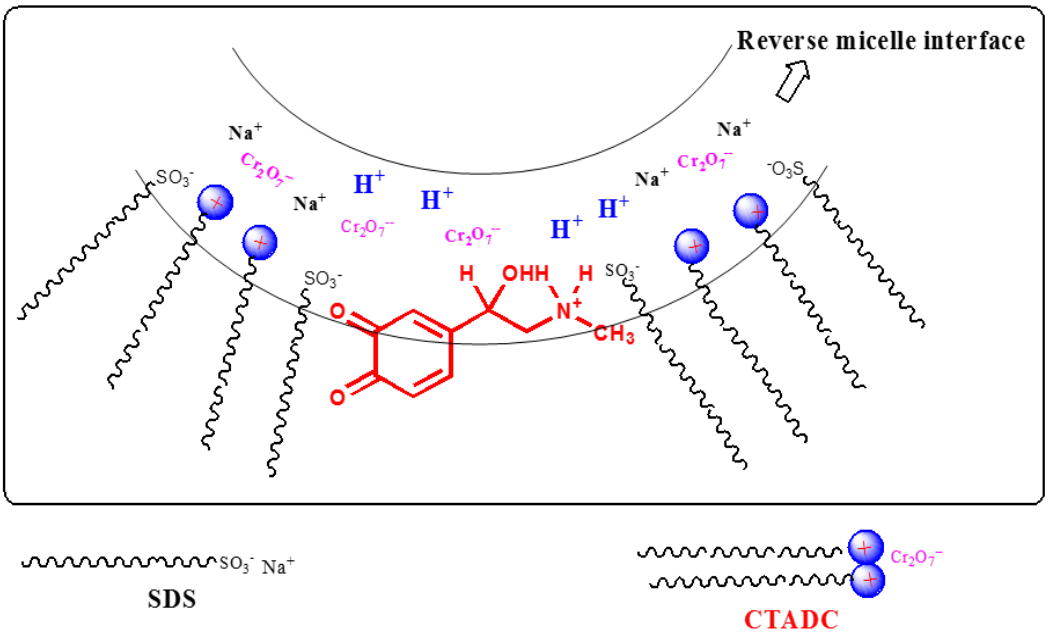


Figure 3.4 Effect of [surfactant] on the rates of the reaction in the oxidation of epinephrine by CTADC at 298 K.

3.3.3 Effect of temperature

The thermodynamic parameters such as E_a, $\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$, and $\Delta S^{\ddagger}$ were calculated using the Arrhenius and Eyring equation, and the results are presented in Table 3.2. The high-negative values of $\Delta S^{\ddagger}$ support the involvement of a highly ordered transition state than the reactants as discussed in Chapter 2. Thus, formation of a cyclic or a forced transition state may be proposed for the abstraction of *o*-hydroxyl proton of complex C2 to form adrenochrome in the rate-determining step.[41]



(Scheme 3.3)

3.3.4 Effect of solvent polarity

The proposed mechanism especially formation of forced nonpolar transition state is further supported by the effect of solvent polarity on the reaction rate. The oxidation reaction was carried out in solutions containing varying proportions of acetonitrile-DMSO in presence of acetic acid. The observed rate constants (Table 3.3) were correlated with different solvent parameters such as dielectric constant (ε), π^* (solvent polarity), β (hydrogen bond acceptor basicity), A (anion solvating power of the solvent), B (cation solvating power of the solvent) and log P (logarithm of partition coefficient between octanol and water referred as solvent hydrophobicity).

The polarity parameters for the solvent mixtures have been estimated approximately from the polarity parameters of the pure solvents.

Table 3.2 Observed rate constants at different temperatures and activation parameters for the oxidation of epinephrine by CTADC in presence of acetic acid.

Solvent	10^4 k_{obs} (s^{-1})							
	288K	293K	298K	303K	308K	313K	318K	323K
CH_3CN	23.5 ±1.1	32.8 ±1.5	43.1 ±2.2	56.4 ±2.8	67.0 ±3.4	-	-	-
CH_3CN: DMSO (1:1)	-	4.8 ±0.2	6.3 ±0.3	8.6 ±0.4	12.9 ±0.5	20.0 ±0.5	25.6 ±1.1	-
DMSO	-	-	0.4 ±0.02	0.6 ±0.04	1.1 ±0.07	1.8 ±0.1	3.0 ±0.2	5.1 ±0.3

Solvent	E_a kJ mol^{-1}	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta G^{\neq}$ kJ mol^{-1}	$\Delta S^{\neq}$ J mol^{-1} K^{-1}
CH_3CN	39.0	36.5	82.6	-168
CH_3CN: DMSO (1:1)	54.0	51.5	90.6	-133
DMSO	82.5	79.9	98.1	-62

With an increase in polarity of the medium the rate constant is found to decrease, delineating less polar transition state than the reactants (Table 3.3). Figure 3.5 and 3.6 show the change in k_{obs} with change in the composition of DMSO and change in hydrogen bond acceptor (HBA) basicity β of the solvent in acetonitrile-DMSO mixture, respectively. On increasing the mole fraction of DMSO from 0 to 0.2 in acetonitrile/DMSO mixture, a sharp decrease in rate constant is observed (from 43 x 10^{-4} to 9 x 10^{-4} s^{-1}). On the other hand, at higher mole fraction of DMSO (> 0.2), the rate constant is virtually independent of the composition. This observation demonstrates preferential solvation of reactants and/or transition state in DMSO. The validity of isokinetic relationship with an isokinetic temperature of 366K confirms

that the bilinear decreasing trend is not due to a change in mechanism.[42,43] Further, as DMSO contains nucleophilic oxygen, the decrease in rate may also be attributed to nucleophilic role of oxygen of DMSO instead of nitrogen in step 3 of the proposed mechanism (Scheme 3.1). To investigate the matter, the effect of composition of nonpolar solvent like chloroform on the rate constant was also investigated. Similar type of increase in rate constant with decrease in polarity was observed when the reaction was carried out in acetonitrile-chloroform mixture (Table 3.4, Figure 3.7). Thus, it may be proposed that, the change in rate constant with change in solvent polarity is primarily due to the preferential solvation of reactants in polar solvents.

Table 3.3 Observed rate constants in the oxidation of epinephrine by CTADC at 298K in acetonitrile: DMSO solvent mixture.

Mole fraction (CH_3CN)	Mole fraction (DMSO)	$\pi*$	β	*logp*	ε	$10^4\ k_{obs}(s^{-1})$
0.83	0	0.707	0.308	-0.283	31.174	43.1 ± 2.4
0.82	0.01	0.708	0.313	-0.296	31.265	30.2 ± 1.7
0.76	0.07	0.713	0.333	-0.352	31.638	15.2 ± 0.8
0.71	0.11	0.718	0.349	-0.395	31.928	12.2 ± 0.6
0.68	0.14	0.721	0.360	-0.424	32.127	10.2 ± 0.6
0.60	0.22	0.728	0.388	-0.500	32.641	8.8 ± 0.5
0.51	0.30	0.736	0.418	-0.581	33.183	7.7 ± 0.4
0.42	0.39	0.745	0.450	-0.665	33.750	6.3 ± 0.4
0.18	0.61	0.767	0.533	-0.89	35.27	2.4 ± 0.2
0.00	0.78	0.784	0.595	-1.06	36.40	0.4 ± 0.02

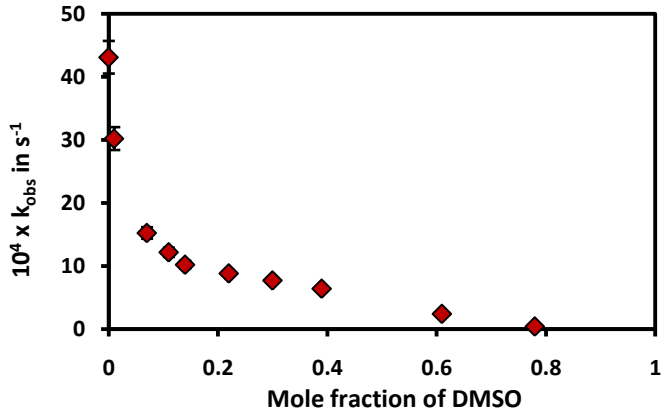


Figure 3.5 Change in k_{obs} with change in the composition of DMSO in acetonitrile-DMSO mixture in the oxidation of epinephrine by CTADC at 298 K.

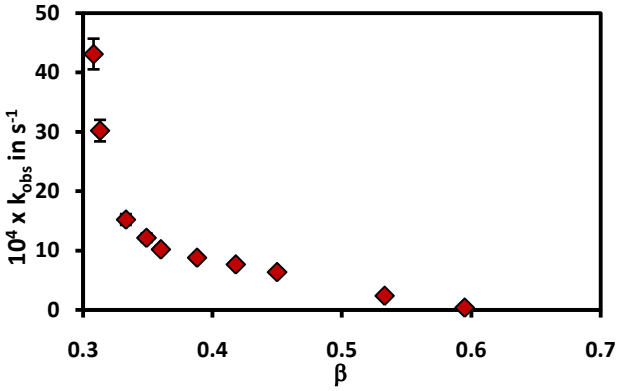


Figure 3.6 Change in k_{obs} with change in β of the acetonitrile-DMSO solvent mixture in the oxidation of epinephrine by CTADC at 298 K.

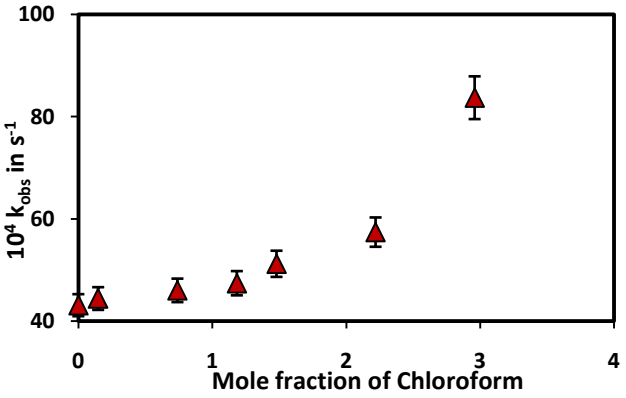


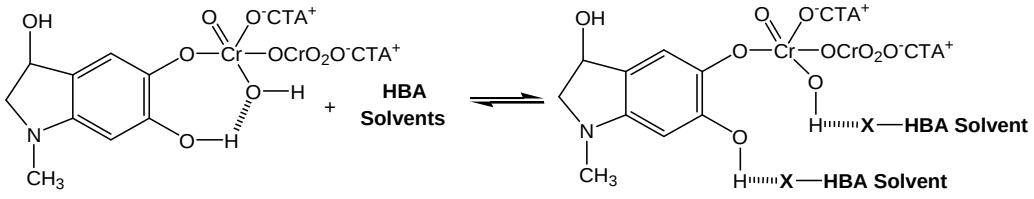
Figure 3.7 Change in k_{obs} with change in the composition of chloroform in acetonitrile: chloroform mixture in the oxidation of epinephrine by CTADC at 298 K.

Table 3.4 Observed rate constants for the oxidation of epinephrine by CTADC at 298K in acetonitrile- chloroform solvent mixture.

Mole fraction (Chloroform)	10^4 $k_{obs}(s^{-1})$
0	43.1 ± 2.5
0.148	44.4 ± 2.5
0.74	46.0 ± 2.6
1.184	47.4 ± 2.8
1.48	51.2 ± 3.1
2.22	57.4 ± 3.5
2.96	83.7 ± 4.8

The decrease in rate constant with an increase in DMSO composition may be attributed to the following reasons. If the reactants are solvated more efficiently in DMSO than acetonitrile with respect to the activated complex, then E_a will increase. Consequently, k_{obs} will decrease and reaction will be slower. Further, if the activated complex is solvated less efficiently in DMSO than acetonitrile compared to the reactants, then E_a will also increase in value leading to slower rate of the reaction. Though both DMSO and acetonitrile are dipolar aprotic solvents with minor difference in the polarity (ε of DMSO= 46.45; ε of acetonitrile= 37.5), there is a large difference in the HBA basicity (β of DMSO =0.76; β of acetonitrile =0.31). Thus, the preferential solvation of the reactants (the oxidant-substrate complex **C2** in the rate-determining step) is supposed to be due to specific solute-solvent interaction, that is, hydrogen bonding. This proposal also gets support from similar decrease of rate constant with respect to β (Figure 3.6). In the oxidant-substrate complex **C2**, two types of hydrogen bonding leading to difference in rate are expected (i) intramolecular H-bonding in low polar and low HBA solvents, (ii) intermolecular H-Bonding with higher HBA solvents (Scheme 3.4). From Scheme 3.4 it is obvious that, stronger intramolecular H-bonding leads to higher rate of hydrogen abstraction from the o-hydroxy group, and thus the rate of the reaction is higher. High polar solvents having

higher β values preferentially solvate the complex and also stabilize the complex through intermolecular H-bonding, leading to lesser rate of decomposition of the complex. The higher energy of activation and slower rate of formation of adrenochrome with an increase in DMSO composition (Table 3.2) is thus attributed to the stronger intermolecular hydrogen bonding providing additional stabilization to oxidant-substrate complex. The high polar nature of DMSO may also destabilize the nonpolar transition state compared to acetonitrile. This phenomenon is presented graphically in Scheme 3.5. The lower the temperature is, the stronger is the preferential solvation of the probe molecules by the molecules of solvent.[44] An increase in temperature results in increased kinetic energy of molecules, and thereby the interaction between molecules connected with preferential solvation becomes weaker. The hydrogen bonding between the reactants and solvents also weakens, resulting in an increase in the rate of reaction.



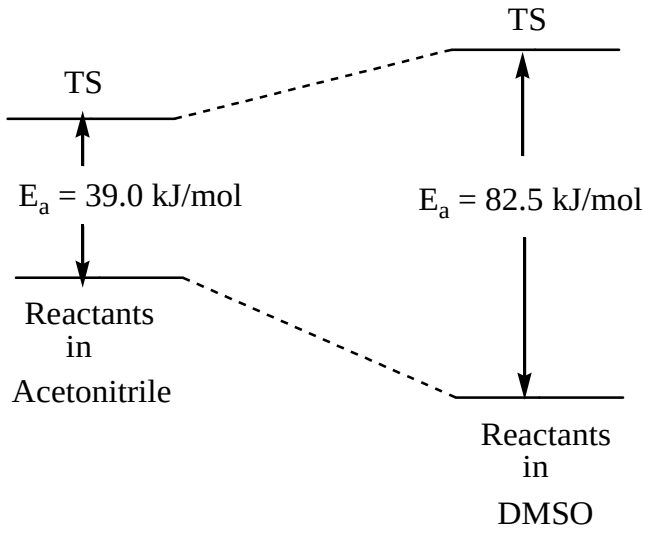
Intramolecular H-Bonding in nonpolar/low polar solvents

Intermolecular H-Bonding with high HBA solvents

X: electronegative atom in HBA solvents

(Scheme 3.4)

The proposition of the formation of a nonpolar transition state compared to that of the reactant is also supported from the numerical values of entropy of activation. The entropy of activation ($\Delta S^{\neq}$) in acetonitrile is -168 J mol^{-1} K^{-1} while in DMSO it is found to be – 62 J mol^{-1} K^{-1}. The values of $\Delta S^{\neq}$ are generally influenced by the degree of solvation and solvent polarity. If the transition state is more extensively solvated than the reactants, $\Delta S^{\neq}$ assumes a larger negative value due to appreciable increase in ordering in the solvation shell.[43] Thus, the higher negative value of $\Delta S^{\neq}$ in acetonitrile as compared to DMSO may be attributed to the greater solvation of the nonpolar transition state in the less polar solvent.



(Scheme 3.5)

3.4 CONCLUSION

The oxidant (CTADC) oxidizes epinephrine to adrenochrome selectively without affecting the secondary hydroxyl group present in the side chain of the epinephrine. The reaction proceeds through a multi-step ionic mechanism. From the experimental data a mechanism was proposed where epinephrine is first converted to epinephrine quinone. Intramolecular cyclization followed by aromatization through proton transfer affords leucochrome which further oxidizes to the product adrenochrome through the rate-determining decomposition of complex **C2** via a less polar transition state. The proposed mechanism gets support from the rate- retarding effect in presence of acetic acid and surfactant. CTADC assembles to form reverse micelles in nonpolar solvents with cations at the interface providing different residing sides for the reactants thus mimicking enzyme activity. The substrate and oxidant are partitioned into two different types of environments, and accordingly the reaction is controlled. The outcome from solvent effect illustrates that, in the rate- determining step the oxidant-substrate complex decomposes via a cyclic nonpolar transition state. Polar solvents with higher HBA ability stabilize the reactants and destabilize the transition state leading to an increase in energy of activation and lesser rate of reaction.

3.5 REFERENCES

1. Blaschko, H. *Br Med. Bull.* **1973**, *29*, 105.
2. Furchgott, R. F. *Pharmacol. Rev.* **1959**, *11*, 429.
3. Rizza, R. A.; Cryer, P. E.; Haymond, M. W.; Gerich,J. E. *J. Clin. Invest.* **1980**, *65*, 682.
4. Minneman, K. P.; Pittman, R. N.; Molinoff, P. B. *Annu. Rev. Neurosci.* **1981**, *4*, 419.
5. Green, D. E.; Richter, D. *Biochem. J.* **1937**, *31*, 596.
6. Mazcr, A.; Green, S.; Shorr, E. *J Biol. Chem.* **1956**, *220*, 227.
7. Green, S.; Mazur, A.; Shorr, E. *J. Biol. Chem.* **1956**, *220*, 237.
8. Slater, E. C. *Biochem. J.* **1949**, *44*, 305.
9. Trautner, E. M.; Bradley, T. R. *Australian J. Sci. Res.* **1951**, *4*, 303.
10. Chaix, P.; Pallaget, C. *Biochim. Biophys Acta.***1953**, *10*, 462.
11. Falh, J. E. *Biochem. J.* **1949**, *44*, 369.
12. Borg, D. C. *Proc. Nat. Acad. Sci. U.S.A.* **1965**, *53*, 633.
13. Behonick, G. S.; Novak, M. J.; Nealley, E. W.; Baskin, S. I. *J. Appl. Toxicol.* **2001**, *21*, S15.
14. Walaas, E.; Walaas, O. *Biochem. Biophys.* **1961**, *95*, 151.
15. Walaas, E.; Walaas, O.; Haavaldsen, S. *Arch. Biochem. Biophys.* **1963**, *100*, 97.
16. Yoshioka, M., Kirino, Y., Tamura, Z., and Kwan, T. *Chem. Pharm. Bull.* **1977**, *25*, 75.
17. Plancheral, D.; von Zelewsky, A. *Helv. Chim. Acta.* **1982**, *65*, 1929.
18. Tao, Z.; Wang, G.; Goodisman, J.; Asefa, T. *Langmuir* **2009**, *25*, 10183.
19. Szigyarto, I. Cs.; Szabo, L.; Simandi, L.I. *J. Mol. Catal. A* **2013**, *372*, 66.
20. Lupano, L. V. L.; Martínez, J. M. L.; Piehl, L. L.; Rubin de Celis, E.; Campo Dall' Orto, V. *Appl. Catal. A* **2013**, *467*, 342.a
21. Lupano, L. V. L.; Martínez, J. M. L.; Piehl, L. L.; Rubin de Celis, E.; Maria Torres Sanchez, R.; Campo Dall' Orto, V. *Langmuir* **2014**, *30*, 2903.
22. Beaupre, B. A.; Carmichael, B. R.; Hoag, M. R.; Shah, D.D.; Moran G. R. *J. Am. Chem. Soc.* **2013**, *135*, 13980.
23. Hoellein, L.; Holzgrabe, U. *Int. J. Pharm.* **2012**, *434*, 468.
24. Exner, O. *Coll. Czech. Chem. Commun.* **1964**, *29*, 1094.
25. Hegde, R. N.; Shetti, N. P.; Nandibewoor, S. T. *Ind. Eng. Chem. Res.* **2009**, *48*, 7025.
26. Jeffery, G. H.; Bassett, J.; Mendham, J.; Denny, R. C. Vogel's text book of quantitative chemical analysis, 5th addition, ELBS Longman, U. K. 1996, P370.

27. West, G. B. *J. Pharmacol.* **1947**, *2*, 121.

28. Misra, H. P.; Fridovich, I. *J. Biol. Chem.* **1972**, *247*, 3170.

29. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006**, *71*, 3522.

30. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006**, *71*, 6759.

31. Patel, S.; Mishra, B. K. *Int. J. Chem. Kinet.* **2006**, *38*, 651.

32. Garnayak, S.; Patel, S. *Ind. Engg. Chem. Res.* **2013**, *2*, 13645.

33. Patel, S.; Sung, D. D.; Mishra, B. K. *Indian J. Chem. A.* **2008**, *47A*, 1218.

34. Heacock, R. A. *Chem. Rev.* **1959**, *59*, 181.

35. Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochmann, M. In Advanced inorganic chemistry, 6th ed.; John Wiley and Sons: New York, 1999; p 747.

36. Yalgudre, R. S.; Gokavi, G. S. *Ind. Eng. Chem. Res.* **2012**, 51, 5135.

37. Hegde, R. N.; Shetti, N. P.; Nandibewoor, S. T. *Ind. Eng. Chem. Res.* **2009**, *48*, 7025.

38. Hiremath, D. C.; Hiremath, C. V.; Nandibewoor, S. T. *Eur. J. Chem.* ***2006***, 3, 13.

39. Rahman, M.; Rocek, J. *J. Am. Chem. Soc.* **1971**, *93*, 5462.

40. Saraswat, S.; Sharma, V.; Banerji, K. K. *Proc. Indian. Acad. Sci. Chem. Sci.* **2003**, *115*, 75.

41. Braisted, A. C.; Schultz, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 7431.

42. Leffler, J. E. *J. Org. Chem.* **1955**, *20*, 1202.

43. Ruff, F.; Csizmadia, I. G. Organic reactions, Equillibria, kinetics and mechanism, Elsevier, New York, 1994.

44. Jozwiak, M.; Madej-Kielbik, L.; Piekarski, H. *Thermochimica. Acta.* **2013**, *568*, 122.

Chapter 4

Oxidation of Isoniazid by Cetyltrimethylammonium Dichromate

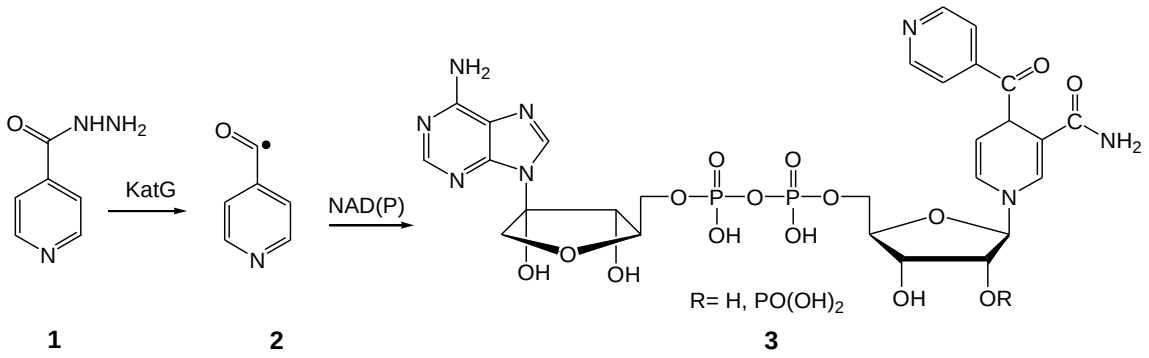
4.1 INTRODUCTION

Tuberculosis (TB) is one of the deadly infectious diseases caused by *Mycobacterium tuberculosis* (Mtb) and is responsible for over two million deaths annually.[1] TB is more widespread in the world today than never before due to the emergence of multiple drug resistant tuberculosis (MDR-TB), extensively-drug-resistant TB (XDR-TB) and HIV associated TB.[2-4] The treatment of this disease is one of the biggest challenges to the scientific community.

Isoniazid (INH) is selected as one of the most effective inhibitors of bacteria *Mycobacterium smegmatis* and Mtb, and widely used as a first line drug along with rifampicin and streptomycin for the chemotherapy of tuberculosis since 1952.[5,6] Soon after the reemergence of tuberculosis in the mid-1980s coupled with increasing numbers of INH-resistant bacterial strains[7-9] several research groups throughout the world are engaged in developing novel, highly effective, fast acting anti-TB drugs with low toxicity profiles and performing activity against both drug-sensitive and drug resistant Mtb. Though several probable TB drug candidates are synthesized and screened for their antimycobacterial properties, none of these antimycobacterials are suitable and approved for tuberculosis treatment till today.[10-15] Therefore, it becomes desirable to investigate the chemistry and the mode of action of this drug in diverse environment, which will help to understand drug resistance.

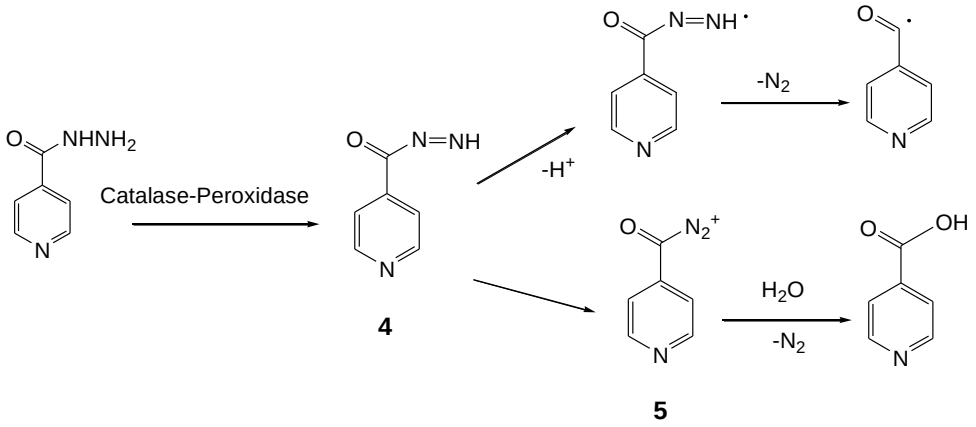
INH (**1**) is a pro-drug[16-19] activated by the enzyme catalase-peroxidase KatG[20-26] to generate the active isonicotinoyl radical (**2**)[27-29] which is responsible for the lethal effect on bacterial cells.[30-33] **2** forms covalent linkage with the nicotinamide moiety of NAD and NADP coenzymes of the reductases InhA[31-32] and MabA[33] respectively to form the true drug **3** (Scheme 4.1), and inhibit the biosynthesis of mycolic acids which is known to be the essential and specific constituents of the mycobacteria cell envelope.[34-36] The formation of the reactive isonicotinoyl radical is found to proceed via an acyl diazene intermediate (**4**) through two electron dehydrogenative oxidation

process. One electron oxidation of **4** followed by loss of proton and liberation of dinitrogen generates the acyl radical (Scheme 4.2).[28, 37]



1 **2** **3**

(Scheme 4.1)



4 **5**

(Scheme 4.2)

In the mechanistic studies of enzymic oxidation of isoniazid by the catalase-peroxidase of Mycobacterium tuberculosis, Johnsson and Schultz reported the involvement of an electrophilic pathway, where the acyldiazene intermediate is further oxidized to acyldiazoniumion (**5**) which then reacts with water or amine to form isonicotinic acid or isonicotinamide respectively (Scheme 4.2).[18] These results suggested that isoniazid is oxidized by the catalase-peroxidase *in vivo* into highly reactive radical and electrophilic species and target the essential enzyme of Mycobacterium tuberculosis through covalent irreversible modification of the protein.

The activation/oxidation of isoniazid by various oxidizing agents such as hydrogen peroxide, bromate, Mn^{2+}, potassium permanganate, potassium ferricyanide, N-haloarenesulfonamidates, diperiodatocuprate(III), quinolinium dichromate, etc. have been undertaken mostly in the aqueous medium in the absence or in the presence of enzyme catalase-peroxidase to observe the effect of these oxidizing species towards the bio-activation of isoniazid.[38-45] However, to the best of our knowledge the oxidation of isoniazid by Cr(VI) in a hydrophobic biomimetic environment has never been elucidated. Many biological processes occur at membrane surfaces or within their hydrophobic moiety. Owing to the presence of ionic head groups and hydrophobic tail of the lipids, biological membrane shows different binding properties of charged and uncharged forms of molecules such as drugs. Among the membrane models utilized, surfactant systems can be considered as an interesting alternative to study various biological processes because of their amphiphilic properties like lipids and of the relative simplicity of these systems, and therefore have been used with this purpose.[46]

Objectives: In this context, we have made an attempt to investigate the kinetics and mechanism of oxidative metabolism of antitubercular drug isoniazid by CTADC using UV-vis spectroscopic method. CTADC was used for the purpose due to its surface active properties to form various organized assemblies.[47-51] Specific objectives of the present section are outlined below.

- To identify the products of oxidative metabolism of isoniazid by CTADC to observe the chemoselectivity and mildness of the reaction process.
- To establish the mechanism of the oxidative degradation and to compare it with that of biological and biomimetic systems.
- To establish a correlation between reaction parameters to that of the rate and mechanism of the reaction.

4.2 EXPERIMENTAL

4.2.1 Materials

Cetyltrimethylammonium dichromate (CTADC) was prepared by the method reported earlier[49] as elaborated in chapter 2 and its purity was checked by estimating Cr (VI) iodometrically.[52] Isoniazid (Merck, India) was purified by recrystallization from ethanol. Glacial acetic acid (Merck, India) was used without further purification. The organic solvents were purified by the standard methods.[53] CTAB and SDS were purified by recrystallization from aqueous ethanol. TX-100 (Merck, India) was used without further purification.

4.2.2 Product analysis

The reaction mixture containing CTADC (1.72gm, 2M) and isoniazid (0.15gm, 1M) in chloroform in presence of 10% acetic acid was stirred and refluxed at 50°C for 3 hours. The mixture turned green indicating the formation of Cr(III). The completion of the reaction was monitored by TLC. The solvent was evaporated and the reaction mixture was subjected to column chromatography. The column chromatographic separation was done in a classical glass column using silica gel (100-200 Mesh) as adsorbent and chloroform-methanol (9:1) mixture as eluent. A white solid product thus obtained (86 mg, yield 64%, Melting Point 305 ^{o}C (sublimes)) was characterized from IR, NMR and CHNS analysis. IR (KBr, $\nu max/cm^{-1}$): 2924 (C-H str), 2407 (O-H str), 1712 (C=O str) 1415 (C=N str) (Figure 4.1). ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 3.47 (bs), 8.76 (2H, d, J = 3.6 Hz), 7.8 (2H, d, J = 3.6 Hz) (Figure 4.2). ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm 166.96, 150.18, 140.1, 129.3 (Figure 4.3). Anal. Calcd. for $C_6H_5N_1O_2$: C, 58.54; H, 4.06; N, 11.38. Found: C, 58.12; H, 4.04; N, 11.34. The IR, NMR and CHNS data analysis of the product agreed well with that of isonicotinic acid. In absence of acetic acid the oxidation reaction was completed after about six hours. The product in this case was also found to be isonicotinic acid.

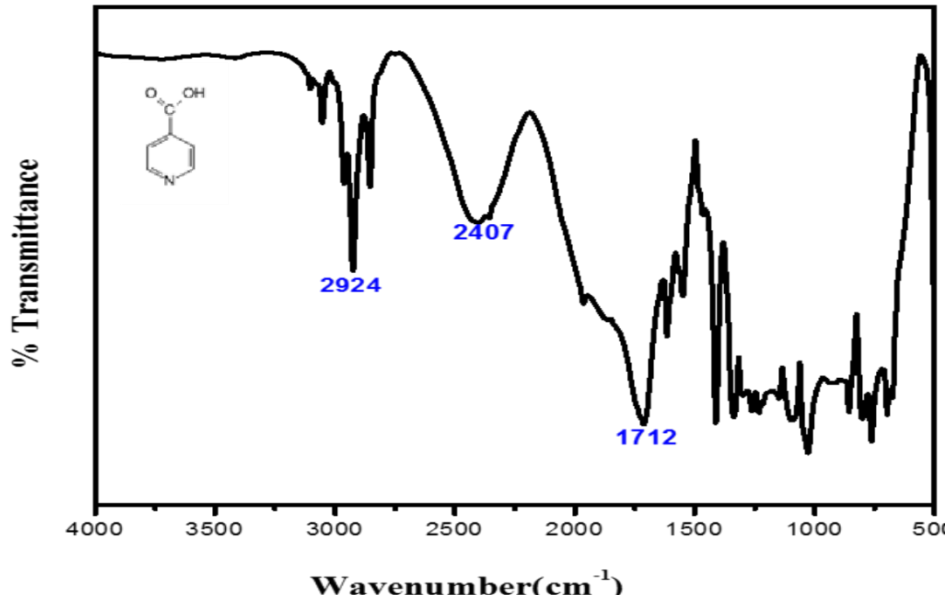


Figure 4.1 IR Spectrum of isonicotinic acid

4.2.3 Kinetic measurements

To study the kinetics of oxidation, conventional Uv-vis spectroscopic technique was used using Shimadzu (UV-1800) UV-vis spectrophotometer. The temperature in the reaction cell was controlled by circulating water by using Lauda thermostat within a temperature fluctuation of 0.05^{o}C. The reactions were performed under pseudo-first-order conditions by keeping excess of the isoniazid with respect to CTADC. The rate of disappearance of the Cr(VI) species was followed spectrophotometrically by monitoring the absorption band at 380 nm in the absence of acid and 450 nm in presence of acetic acid. The first-order rate constant, k_{obs}, was obtained from the linear plot of log [CTADC] against time for up to 75% completion of the reaction. The values reported are the average of at least triplicate runs and were reproducible within 6% error. The solvent kinetic isotope effect was investigated using 1:1 mixtures of H_2O-acetonitrile, D_2O-acetonitrile instead of chloroform in presence of 2% acetic acid.

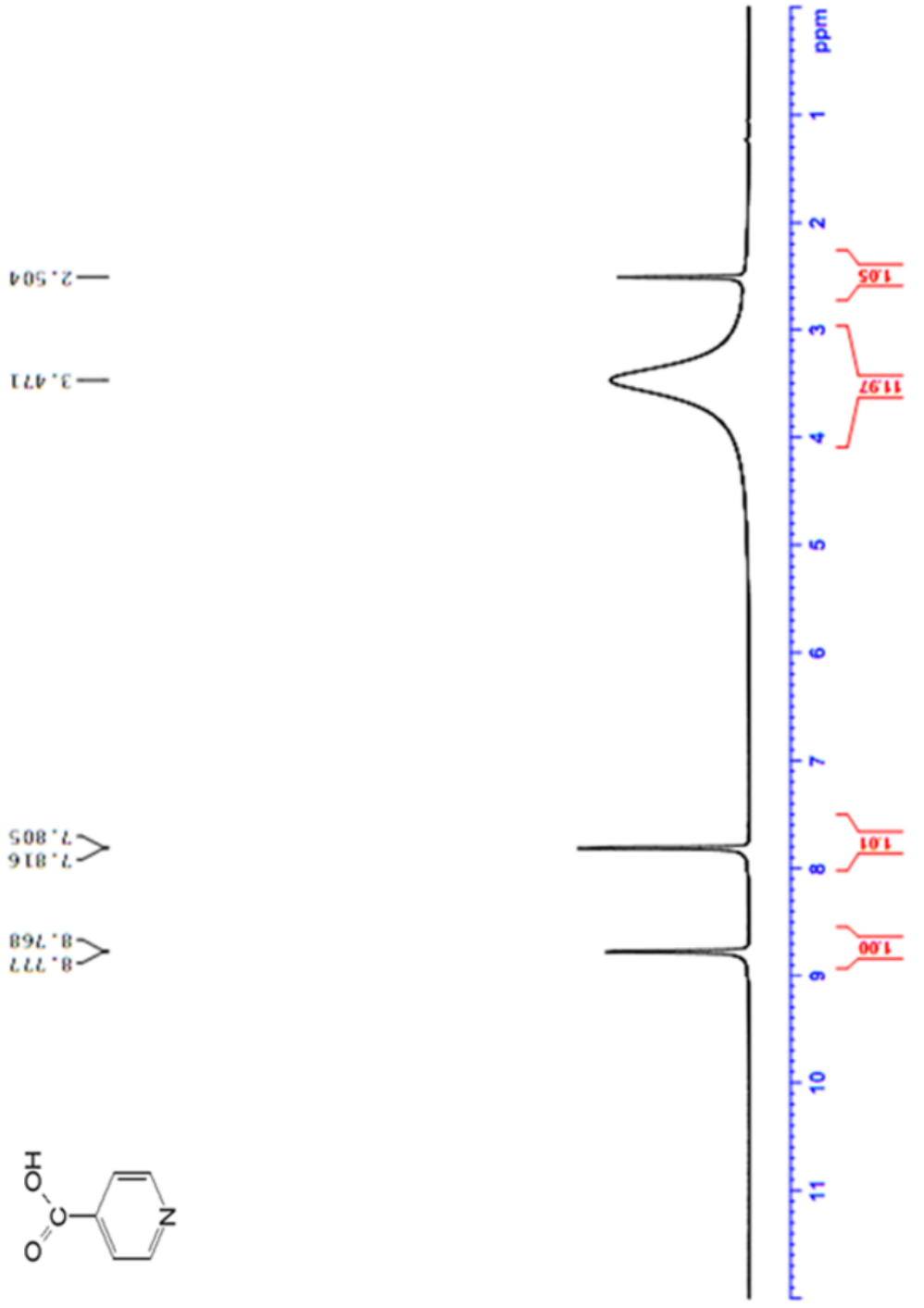


Figure 4.2 ^{1}H-NMR spectrum of isonicotinic acid.

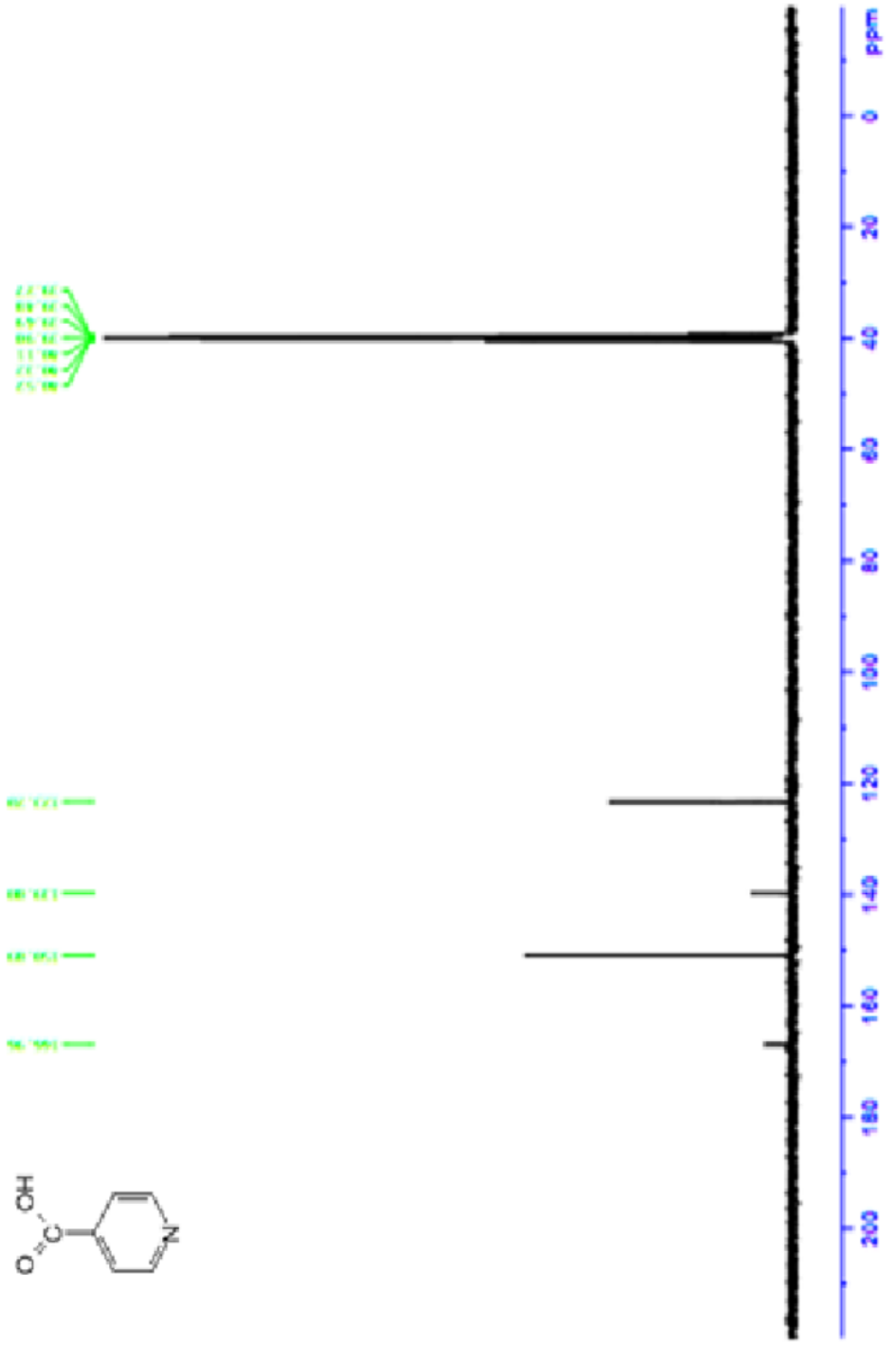


Figure 4. 3 ^{13}C- NMR spectrum of isonicotinic acid.

4.2.4 Stoichiometry

The stoichiometry of the reaction was determined by performing the experiment at 298 K, under the conditions of [CTADC] >> [isoniazid] ([CTADC] = 1.75 x 10^{-4} to 8.73 x 10^{-4} and [Isoniazid] = 4.36 x 10^{-5} to 1.1 x 10^{-4}) at varying isoniazid concentrations. The disappearance of Cr(VI) was followed until the absorbance values became constant. The [CTADC] was estimated after 48 h. A stoichiometry ratio, Δ[CTADC]/Δ[Isoniazid] = 0.7, was observed, which confirmed a 1:1.5 CTADC/isoniazid relationship.

4.3 RESULTS AND DISCUSSION

The reaction mixture consisting of CTADC and isoniazid in chloroform become green after six hours under reflux condition. The oxidation product was isolated using chromatographic separation techniques. From the CHNS, IR and NMR spectral analysis, the product was found to be isonicotinic acid. In presence of 10% acetic acid the oxidation reaction was found to complete after about three hours giving rise to the same product.

Stoichiometric analysis reveals the reaction of 1 mole equivalent of CTADC with 1.5 mole equivalent of isoniazid. The green coloration of the product mixture due to the absorption maxima around 550-580 nm supports the formation of reduced Cr(III).[54] One electron oxidation giving rise to free radicals may not be possible for the present reaction due to the following factors (i) oxidation of isoniazid, in an atmosphere of nitrogen, failed to induce polymerization of acrylonitrile[55] (ii) the addition of acrylonitrile did not affect the rate of oxidation reaction[56] and (iii) absence of signal in the EPR spectrum. Further, following the procedure of Braslau et al.[37] the reaction was carried out in presence of 2,2,6,6-Tetramethylpiperidinyloxy (TEMPO), a stable nitroxyl radical. Absence of any acyloxyamine products may confirm the absence of isonicotinoyl radical in the reaction process. Thus, it may be proposed that, during the oxidation process Cr(VI) is reduced to Cr(IV), which further changes to Cr(III) by a disproportionation reaction in a sequential manner. Using the result, the stoichiometric equation may be formulated as in equation 1.

$2\ (CTA^+)_2\ Cr_2O_7^{--}\ +\ 3$ Isoniazid $+\ 4\ H^+$

$$\rightarrow 3 \text{ Isonicotinic acid} + 3\ N_2 + 5\ H_2O + 4\ CTA^+ + 4\ Cr(III) \qquad (1)$$

For the mechanistic analysis of the oxidation reaction of isoniazid by CTADC, the reaction kinetics was investigated using UV-vis spectroscopic technique. The rates of reactions were monitored by observing the rate of depletion of absorption peak at 450 nm. The observed rate constants, k_{obs} at varying concentration of the substrates involved were calculated maintaining pseudo first order condition and tabulated in Table 4.1.

4.3.1 Effect of acetic acid concentration

The observed rate constant of the reaction increases linearly with increase in the concentration of acetic acid indicating protonation of dichromate which facilitates the oxidation reaction. The plot of log rate vs. log [acetic acid] depicts a fractional order (i.e. 0.5) dependency with respect to acid. The fractional order dependency with respect to acetic acid is ascribed to the protonation of isoniazid. In the presence of acetic acid, there may be a decrease in the rate of attack of protonated substrate and protonated oxidant affecting the reaction order with respect to acetic acid. The k_{obs} obey the following relationship with [acetic acid].

$$k_{obs} = k_0 + k_{cat}\ [\text{acetic acid}]$$

Where, k_{cat} refers to the rate constant for the acid catalyzed oxidation of isoniazid and k_0 refers to uncatalyzed rate constant and found to be $2.7 \times 10^{-3}\ s^{-1}$ indicating an appreciable oxidation of isoniazid with CTADC in the absence of acid. Thus to have more insight into the reaction mechanism, the reaction was studied both in the absence and in the presence of acetic acid.

Table 4.1 Effect of [Isoniazid], [CTADC] and [Acetic Acid] on the Oxidation of Isoniazid by CTADC in Chloroform at 298 K

[CTADC] x 10^4 M	[Isoniazid] x 10^4 M	[Acetic acid] M	k_{obs} x 10^3 s^{-1}
0.44	11	--	1.81 ± 0.05
0.88	11	--	2.06 ± 0.06
1.31	11	--	2.28 ± 0.07
2.18	11	--	2.69 ± 0.07
2.64	11	--	2.48 ± 0.08
3.05	11	--	2.26 ± 0.08
0.44	11	0.79	6.62 ± 0.27
0.88	11	0.79	6.02 ± 0.24
1.31	11	0.79	5.55 ± 0.22
2.18	11	0.79	4.82 ± 0.17
2.64	11	0.79	4.66 ± 0.17
3.05	11	0.79	4.51 ± 0.16
0.44	1.1	--	1.09 ± 0.03
0.44	2.2	--	1.43 ± 0.04
0.44	2.73	--	1.50 ± 0.03
0.44	3.27	--	1.56 ± 0.04
0.44	3.82	--	1.62 ± 0.05
0.44	4.36	--	1.65 ± 0.06
0.44	4.91	--	1.68 ± 0.06
0.44	5.45	--	1.72 ± 0.05
0.44	8.18	--	1.78 ±0.07
0.44	11.0	--	1.81 ± 0.05

Continued…

[CTADC] x 10^4 M	[Isoniazid] x 10^4 M	[Acetic acid] M	k_{obs} x 10^3 s^{-1}
0.44	2.2	0.79	4.37 ± 0.16
0.44	2.73	0.79	4.69 ± 0.17
0.44	3.27	0.79	5.02 ± 0.20
0.44	3.82	0.79	5.31 ± 0.21
0.44	4.36	0.79	5.54 ± 0.21
0.44	4.91	0.79	5.73 ± 0.23
0.44	5.45	0.79	5.92 ± 0.23
0.44	8.18	0.79	6.40 ± 0.24
0.44	11.0	0.79	6.62 ± 0.26
2.18	11	0.79	4.82 ± 0.17
2.18	11	0.32	3.34 ± 0.12
2.18	11	0.48	3.90 ± 0.13
2.18	11	0.64	4.38 ± 0.15
2.18	11	0.79	4.82 ± 0.17
2.18	11	0.95	5.12 ± 0.20
2.18	11	1.11	5.34 ± 0.20
2.18	11	1.27	5.76 ± 0.23
2.18	11	1.43	6.25 ± 0.25
2.18	11	1.59	6.52 ± 0.25
2.18	11	1.75	6.83 ± 0.27
2.18	11	1.91	7.55 ± 0.28

4.3.2 Effect of isoniazid concentration

The observed rate constant determined in the absence of acetic acid was found to increase asymptotically with increasing concentration of isoniazid, approaching its maximum value of 1.8 x 10^{-3} s^{-1} (Figure 4.4A). Thus a Michaelis-Menten type kinetics is proposed i.e. the reaction involves the binding of oxidant to the substrate to form a complex prior to the rate determining step. The complex subsequently decomposes to the products (equation 2 and 3).

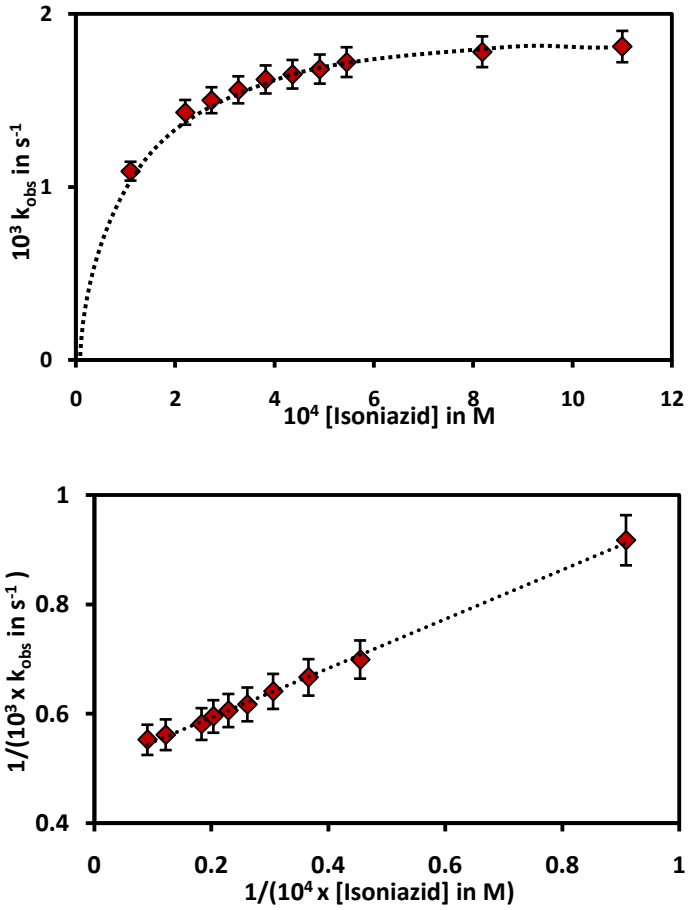


Figure 4.4 (A) Plot of 10^3 x k_{obs} versus 10^4 x [isoniazid] and (**B**) Plot of 1/(10^3 x k_{obs}) versus 1/(10^4 x [isoniazid]) for the oxidation reaction of isoniazid with CTADC in chloroform at 298 K.

$$\text{Isoniazid + Oxidant} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \text{Complex} \qquad (2)$$

$$\text{Complex} \xrightarrow{k_2} \text{Product} \qquad (3)$$

By applying steady-state approximation to equation 2 and 3 the rate expression is obtained (equation 4).[57]

$$\frac{1}{k_{obs}} = \frac{1}{k_2 K[\text{Isoniazid}]} + \frac{1}{k_2} \qquad (4)$$

The steady-state dissociation constant of the oxidant-substrate complex known as the Michaelis-Menten constant, $K_m(= (k_{-1}+ k_2)/k_{+1})$, was calculated to be 0.9×10^{-4} M. K_m is the substrate concentration at which the reaction rate falls to half-maximum, and is an inverse measure of the substrate's affinity for the catalyst (enzyme). Smaller is the K_m, higher is the affinity of the catalyst toward the substrate. K_m values for the oxidation reaction of CTADC with different alcoholic substrates are compared with that of isoniazid (Table 4.2). Though in each of the three alcoholic substrates, chromate ester intermediate is formed with the bonding of hard donor –OH with hard accepter Cr(VI), K_m for cholesterol is much lower than that of cyclohexanol and benzylalcohol. The lower K_m and higher affinity for cholesterol may be attributed to the hydrophobic interaction with the non-polar tail of the lipophilic oxidant.[51] Though isoniazid has less hydrophobicity and softer bonding ($-NH_2$) site, the lower K_m values compared to the above alcoholic substrates show higher affinity of CTADC towards isoniazid. This may be explained through the proximity of carbonyl oxygen and chromium in the oxidant substrate complex which provides additional stabilization.

Table 4.2 K_m values for the oxidation of organic substrates by CTADC

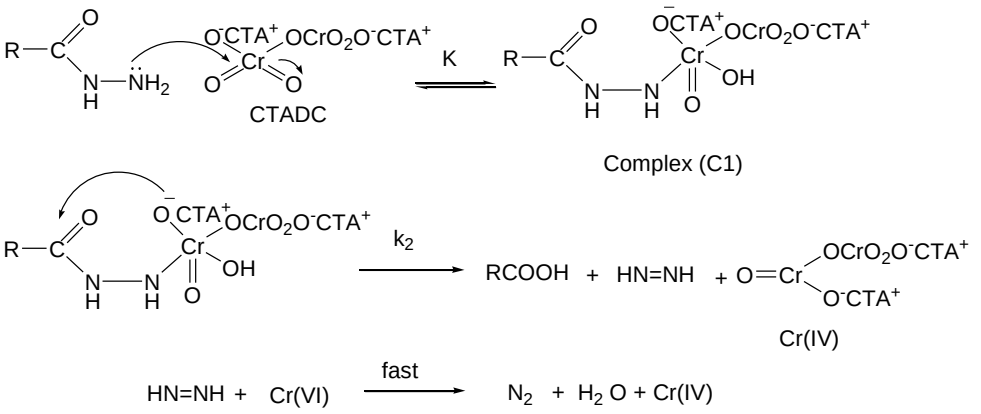
Substrate	K_m (Without acid)	K_m (acid catalyzed)	Refs
Cholesterol	--	2.4×10^{-4} M	51
Benzyl alcohol	--	0.1 M	58
Cyclohexanol	--	0.2M	59
Isoniazid	0.9×10^{-4} M	1.7×10^{-4} M	Present study

From the Lineweaver-Burk double reciprocal plot (Figure 4.4B) k_2 and K ($=k_{+1}/k_{-1}$) were calculated to be 1.99 x 10^{-3} s^{-1} and 11181 dm^3 mol^{-1} respectively. Using K_m, k_2 and K values k_{+1} and k_{-1} were found to be 3.5 x 10^3 $dm^3mol^{-1}s^{-1}$ and 3.15 x 10^{-1} s^{-1} respectively. In the oxidation of isoniazid to isonicotinic acid by the enzyme catalase-peroxidase from Mycobacterium tuberculosis, Johnsson *et al.*[18] have reported similar order of k_{cat} and K_m i.e. 4.5 x 10^{-2} s^{-1} and 1.98 x 10^{-4} M respectively.

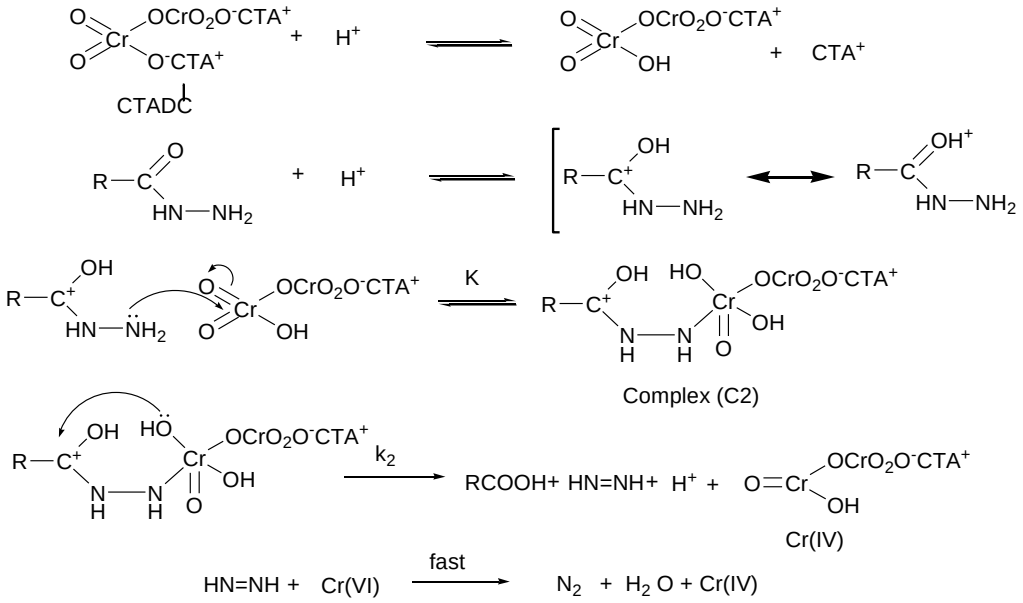
The acid catalyzed process also follows the same trend with change in [substrate]. The corresponding K_m, k_2, K, k_{+1} and k_{-1} are found to be 1.7 x 10^{-4} M, 7.69 x 10^{-3} s^{-1}, 5909 dm^3 mol^{-1}, 10000 dm^3 mol^{-1} s^{-1} and 1.69 s^{-1} respectively. Lower binding affinity of oxidant and substrate in acid catalyzed process may be attributed to the (i) higher polarity of the medium where solvation of isoniazid will be higher leading to a decrease in the interaction of isoniazid and CTADC or (ii) protonation of isoniazid, which reduces the interaction of protonated isoniazid with CTADC. As the actual oxidant is anionic, higher binding affinity of oxidant and protonated isoniazid is expected. However, presence of contact ionpair between dichromate and CTA^+ in nonpolar medium may decrease the binding affinity due to the repulsion between protonated isoniazid and CTA^+.

Consistent with the above observations, the reaction mechanisms for oxidation of isoniazid with CTADC without acid (Scheme 4.3) and in the presence of acetic acid (Scheme 4.4) have been proposed where dichromate ion forms an N-substituted acid hydrazide intermediate complex with isoniazid. The complex subsequently decomposes into products namely isonicotinic acid, diazene and Cr(IV) by a slow rate-determining process, where chromate oxygen attacks to the carbonyl carbon through the formation of a cyclic five membered transition state. Diazene further oxidizes to dinitrogen and water with another mole of Cr(VI). Yalgudre *et al.*[60] while investigating the oxidation of isoniazid by bromate in aqueous hydrochloric acid medium, demonstrated the formation dinitrogen and water from diazene intermediate. The formation of diazene intermediate in the oxidation of isoniazid has also been

reported by Bodiguel *et al*.[61] Karimi *et al*.[62] have described the formation of dinitrogen in the electro-catalytic dehydrogenative oxidation of isoniazid to isonicotinic acid via acyldiazene intermediate **4**.



(Scheme 4.3)



(Scheme 4.4)

Comparing k_{obs}, k_2 and K values of both without acid and acid catalyzed oxidation processes, it is found that the rate of the reaction is dependent mostly on the rate of decomposition of the complex (C). A higher rate of reaction in presence of acid is thus due to higher rate of decomposition of the complex, though the binding affinity of oxidant and substrate is higher in the absence of acid. In the presence of acid, the carbonyl carbon acquires a partial positive charge due to the polarization of

the carbonyl bond owing to the protonation of the carbonyl oxygen. The higher rate of decomposition is thus attributed to the higher electrophilicity of the carbonyl carbon due to which the reactivity of hydroxyl oxygen towards carbonyl carbon increases, which leads to an increase in the overall rate of the reaction. To get further support for the proposed mechanism, effect of temperature, and solvent kinetic isotope effect ($k(H_2O)/k(D_2O)$) have been investigated.

4.3.3 Effect of temperature

The observed rate constant increases linearly with increase in temperature. The thermodynamic parameters such as $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ were calculated from the plot of ln (k/T) vs. 1/T using Eyring equation and are tabulated in Table 4.3. The high-negative entropy of activations is indicative of the formation of a forced, more ordered and extensively solvated transition state than the reactants in the rate-determining step in both the cases.[63,64]

Table 4.3 Observed rate constants and activation parameters for the oxidation of isoniazid by CTADC in the absence of acid and in presence of acetic acid. [Isoniazid] = 1.1 x 10^{-3}M, [CTADC] = 2.18 x 10^{-4} M.

[Acetic acid]	10^3 k_{obs} s^{-1}				$\Delta H^{\ddagger}$ kJ mol^{-1}	$\Delta S^{\ddagger}$ J K^{-1} mol^{-1}	$\Delta G^{\ddagger}$ kJ mol^{-1}
	293K	298 K	303K	308K			
--	1.82 (±0.04)	2.69 (±0.07)	3.7 (±0.15)	4.65 (±0.19)	44.6	-145	87.7
0.79 M	4.26 (±0.15)	4.82 (±0.17)	5.56 (±0.21)	6.1 (±0.23)	15.8	-159	63.2

4.3.4 Determination of solvent kinetic isotope effect

To obtain the solvent kinetic isotope effect ($k(H_2O)/k(D_2O)$), the reaction was carried out in 1:1 mixture of H_2O-acetonitrile and in 1:1 mixture of D_2O-acetonitrile in presence of 2% acetic acid. Acetic acid was equilibrated with H_2O and D_2O for 3 h

before its use in the reaction process. The solvent isotope effect $k(H_2O)/k(D_2O)$ was found to be $((3.3\times10^{-3})/(4.84\times10^{-3}))$ =0.68. The acid catalyzed rate is faster in D_2O than in H_2O, when a pre-equilibrium protonation is involved.[65-67] The inverse solvent kinetic isotope effect i.e. $k(D_2O) > k(H_2O)$, indicates that the NH_2 group is not involved in the rate-determining step and thus supports the formation of complex "C" in the reaction process. Due to immiscibility of chloroform-water binary mixture and improper solubility of reactants in aqueous medium, the solvent kinetic isotope effect is measured in a different solvent medium other than chloroform. Thus it may be anticipated that the reaction mechanism in both the cases differ. The validity of isokinetic relationship (equation 5) for a series of solvents including chloroform and acetonitrile-water mixture was verified from the linear plot of $\Delta H^{\ddagger}$ vs. $\Delta S^{\ddagger}$ (Figure 4.5) and demonstrated the same mechanism for whole solvent series.[68] The isokinetic temperature, β for the present oxidation process in presence of acetic acid is found to be 314 K, where the rate of every member of the series is same.

$$\Delta H^{\ddagger} = \beta \Delta S^{\ddagger} + c \qquad (5)$$

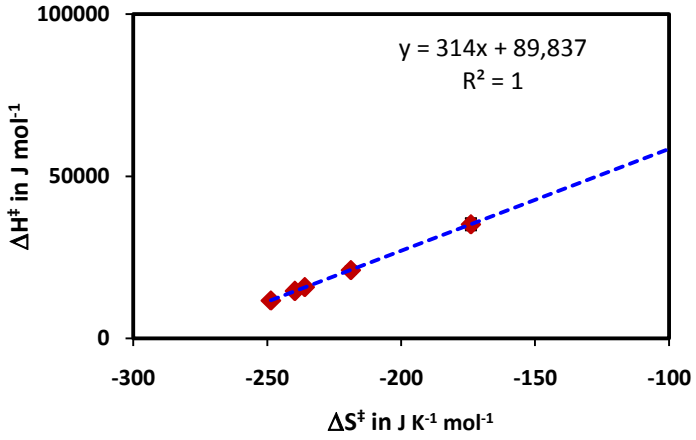


Figure 4.5 Plot of ΔH‡ versus ΔS‡ for the oxidation reaction of isoniazid with CTADC in presence of acetic acid for a series of solvents.

4.3.5 Effect of CTADC concentration

With an increase in [CTADC] in the absence of acid, the rate constants are found to increase up to [CTADC] = 2.18 x 10^{-4} M and then decreases (Figure 4.6A). However, in the presence of acetic acid, direct decrease in rate constant is observed with increase in [CTADC] (Figure 4.6B). These facts demonstrate probable formation of reverse micelles as discussed in previous chapters. The concentration of CTADC at which the decrease in the rate constant begins, differs in the two processes. In presence of acid it starts before 4.4 x 10^{-5} M whereas without acid it is approximately at 2.18 x 10^{-4}M. The presence of acetic acid starts the aggregation at a lower concentration. Amphiphilic molecules form reversed micelle in the non-polar solvents either in presence of water or in presence of non-aqueous polar solvents.[69] The presence of acetic acid triggers the formation of reverse micellar aggregates of CTADC in chloroform and thus the aggregation starts at lower concentration.

Based on the above results, it is proposed that the mechanism of oxidation of isoniazid by CTADC stems on the following;

(i) rate of depletion of complex (C1 or C2) by forward reaction, k_2

(ii) stability of transition state

(iii) the partition of the oxidant and substrate in two micro-heterogeneous domains due to the formation of reverse micelles.

To obtain a clear picture on these aspects, the effect of different surfactants (with varied charge) and the effect of various solvents (with varied hydrophobicity and polarity) on the reactivity were investigated.

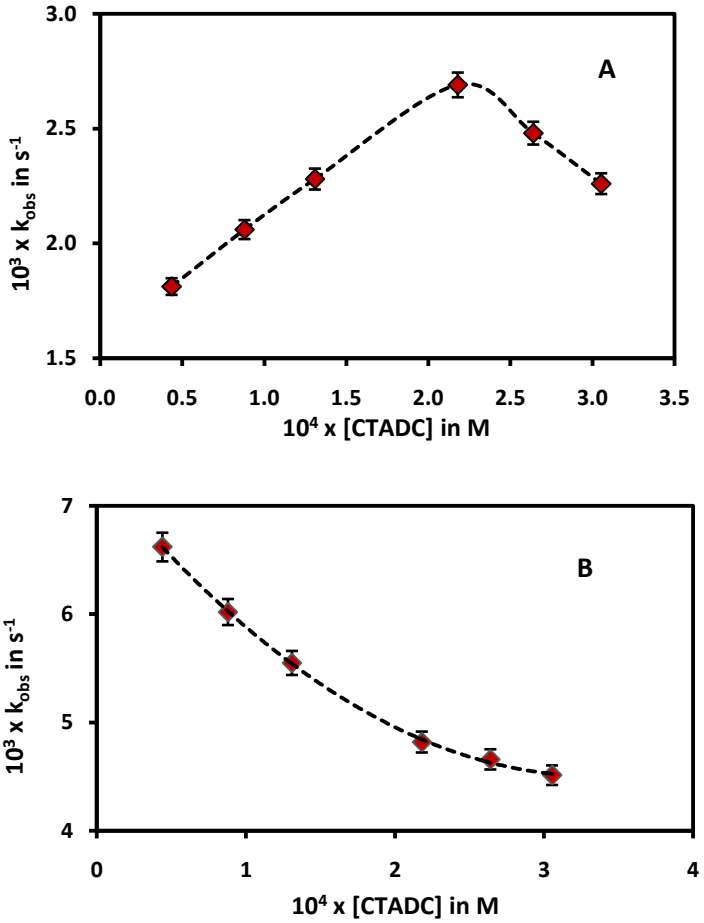


Figure 4.6 Plot of 10^3 x k_{obs} versus 10^4 x [CTADC] in the oxidation reaction of isoniazid with CTADC in chloroform at 298 K. **A:** Without acid **B:** in presence of 0.79M acetic acid. [Isoniazid] = 1.1 x 10^{-3} M

4.3.6 Effect of surfactant

To study the effect of surfactant on the reaction rate CTAB (cationic surfactant), SDS (anionic surfactant) and TX-100 (nonionic Surfactant) were used. All the three surfactants can form reverse micelles in non-polar medium, [70-73] mixed micelle with various ionic and nonionic surfactants[74-78] and also with CTADC. If the rate decrease with increase in [CTADC] is only because of the partition of oxidant and substrate in two different micro-heterogeneous media, consequently a more or less decrease in rate would have been expected upon addition of surfactants to the reaction mixture.

In the absence of acid, a sharp decrease in rate constant was observed on addition of CTAB and SDS which supports the formation of reverse micellar aggregates (Figure 4.7). However, on addition of TX-100 the rate constant initially increased up to a concentration of 1.16 x 10^{-2} M and then decreased sharply (Figure 4.8). Above 3.5 x 10^{-2} M of TX-100 the rate constant almost became stable around 1.2 x 10^{-3} s^{-1}. The increase is explained through a greater attractive interaction of oxy-ethylene units of TX-100 with the cationic head group of CTADC and repulsive interaction with that of anionic oxygen of the complex increasing the rate of decomposition of the complex. At high concentration of TX-100 the partition factor may become more important than the electronic factor in the reverse micellar aggregates of the nonionic surfactant. Assuming the rate decrease at high [surfactant] is due only to the partition effect, for complete entrapment of a CTADC it requires a composition of 1:160 of CTADC/TX-100 and 1:16 of CTADC/SDS in the reaction mixture.

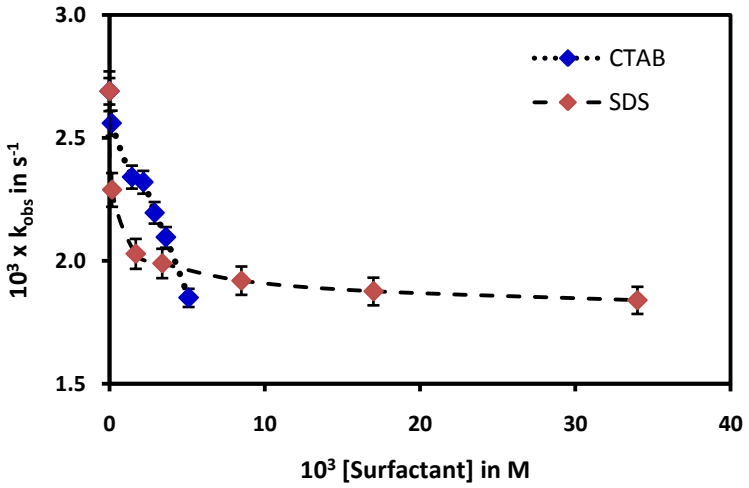


Figure 4.7 Effect of [CTAB] and [SDS] on the rate constant in the oxidation of isoniazid by CTADC in chloroform at 398K. [Isoniazid] = 1.1 x 10^{-3}M, [CTADC]= 2.18 x 10^{-4} M.

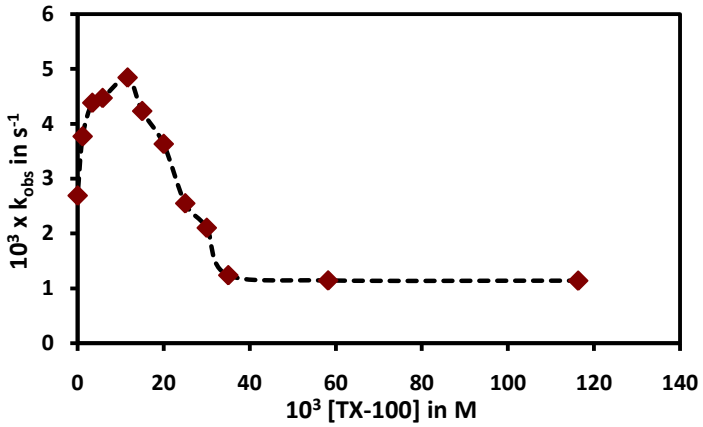


Figure 4.8 Effect of [TX-100] on the rate constant in the oxidation of isoniazid by CTADC in chloroform at 398K. [Isoniazid] = 1.1 x 10^{-3}M, [CTADC]= 2.18 x 10^{-4} M.

In the presence of acetic acid, the three surfactants influence the rate differently. When CTAB was added to the reaction medium, a decrease in the rate constant was observed while addition of SDS increased it (Figure 4.9). The addition of nonionic surfactant TX-100 has no significant effect on the reaction rate in acidic medium. CTAB forms reverse micelle with a cationic interface and thus in presence of acetic acid, apart from the partition factor the decrease in rate constant may be attributed to the following factors (i) decrease in the effective concentration of H^+ on cationic interface of CTAB, (ii) slow formation of the active protonated dichromate and (iii) decrease in the electrophilicity of the carbonyl carbon by reducing the protonation of the carbonyl oxygen on the cationic interface. In contrast to CTAB, the increase in the rate constant in presence of anionic surfactant SDS is explained through (i) an increase in the effective concentration of H^+ on the anionic interface of SDS, (ii) the rapid formation of the active protonated dichromate and (iii) increase in the protonation of carbonyl group thus increasing the electrophilicity of the carbonyl carbon on the anionic interface of SDS reverse micelles. In case of TX-100 the increase in the rate constant due to accumulation of H^+ on the polar oxyethylene interface is compensated by the decrease in the rate constant due to partition of reactants in different phases.

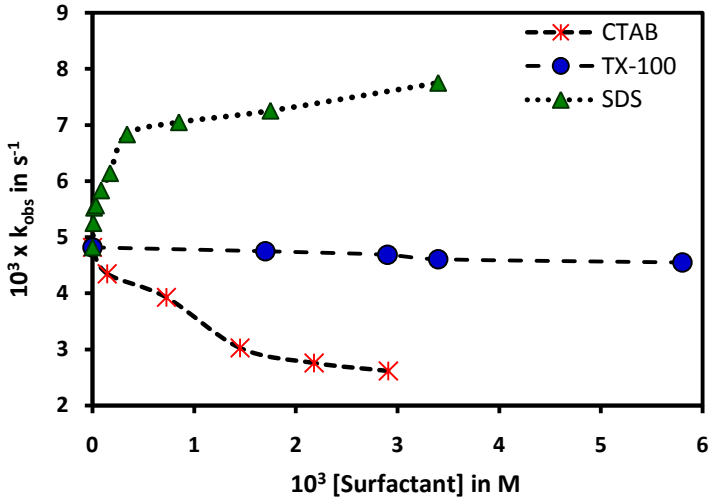


Figure 4.9 Effect of [surfactant] on rate constant in the oxidation of isoniazid by CTADC in presence of acetic acid in chloroform at 398K. [Isoniazid] = 1.1 x 10^{-3}M, [CTADC] = 2.18 x10^{-4} M, [Acetic acid] = 0.79M

4.3.7 Effect of solvent polarity

In the present investigation, twelve different solvents were used having non-polar and dipolar aprotic characteristics. The solvent effect was investigated only in the presence of acid due to poor solubility of reactants in most of the solvents. The observed rate constant shows significant solvent sensitivity (Table 4.4). Herein, we have made an attempt to correlate the solvent induced change in reactivity termed as "solvatoreactivity" with the solvent polarity parameters such as $\pi*$, β, *A*, *B*, *A*+*B*, log *P*, ε, μ, η, ρ and γ. The plots of k_{obs} with various polarity parameters exhibit some interesting results.

The plot of k_{obs} versus polarity parameter $\pi*$ exhibits (Figure 4.10) a negative slope (with benzene, toluene, chloroform and dioxane as outliers), indicating a rate retardation due to an increase in the polarity of the solvents. Further, the plot of dielectric constant (ε) values with k_{obs} (Figure 4.11) shows a sharp rate increase in the lower side (ε : 0 to 9) followed by significant rate decrease in the higher side (ε : 6 to 47). The increase in the rate is due to the solvents like benzene, toluene, chloroform and dioxane, while the rate decrease is due to the solvents DMSO, DMF, acetone,

acetonitrile and nitrobenzene with ethyl acetate, THF and dichloromethane being common in both the cases. The plot of k_{obs} versus Dipole moment (μ) demonstrates similar increasing and decreasing trends (Figure 4.12) in the lower and higher side respectively.

Table 4.4 Observed rate constants for the oxidation of isoniazid by CTADC in various organic solvents in presence of acetic acid at 298K. [Isoniazid] = 1.1 x 10^{-3}M, [CTADC]= 2.18 x 10^{-4} M, [Acetic acid]=0.79 M

Sl. No.	Solvents	10^3 x k_{obs} s^{-1}
1	Toluene	2.63 ± 0.09
2	Benzene	2.37 ± 0.09
3	Chloroform	4.82 ± 0.17
4	Dichloromethane	5.74 ± 0.23
5	Dioxane	4.78 ± 0.20
6	Nitrobenzene	3.86 ± 0.14
7	Tetrahydrofuran	7.23 ± 0.29
8	Acetonitrile	5.08 ± 0.18
9	Ethyl acetate	7.89 ± 0.32
10	Acetone	5.70 ± 0.22
11	Dimethylsulfoxide	1.75 ± 0.05
12	N,N-Dimethylformamide	3.72 ± 0.13

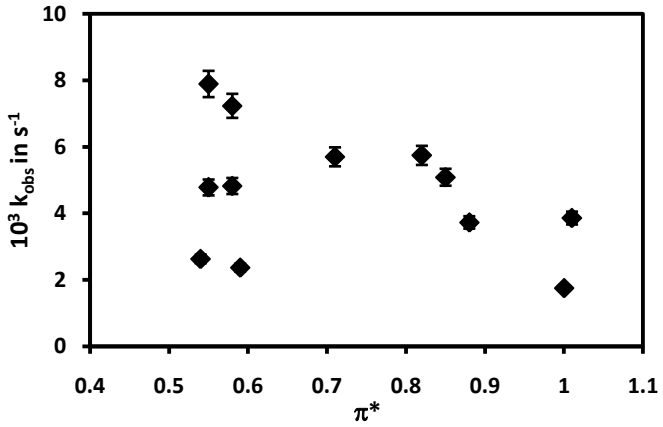


Figure 4.10 Plot of 10^3 x k_{obs} versus $\pi*$ of the solvents in the oxidation reaction of isoniazid with CTADC at 298 K.

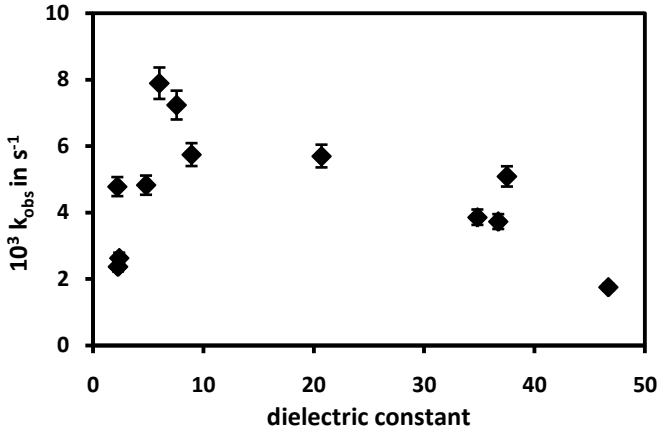


Figure 4.11 Plot of 10^3k_{obs} versus dielectric constant (ε) of the solvents in the oxidation reaction of isoniazid with CTADC at 298 K.

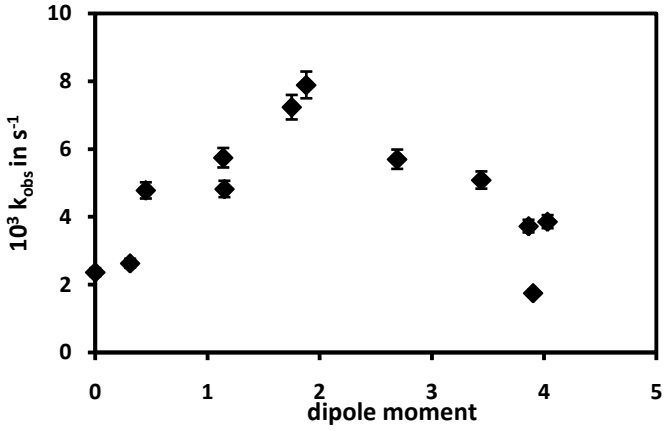


Figure 4.12 Plot of 10^3k_{obs} versus Dipole moment (μ) of the solvents in the oxidation reaction of isoniazid with CTADC at 298 K.

The above results clearly envisage the division of these solvents into two groups, first group for high polar solvents while the second group for apolar solvents. Low polar solvents such as ethylacetate and THF are common for both the groups. For the former polar group, rate of the reaction decreases with increase in polarity, whereas for the later nonpolar and low polar solvent group rate acceleration with polarity is observed. The opposite trends in these two groups of solvents suggest a complex mechanism with varied contribution of these solvents towards the reaction mechanism through the above said three factors.

The negative contribution of polarity towards the reaction rate for the polar solvents suggests a relatively less polar transition state than the reactants. The degree of solvation of polar reactants and the complex (C1 and C2) is more in the polar solvents compared to the transition state. Thus the rate of association of reactants to form complex and also the rate of decomposition of complex (k_2) decreases, leading to overall rate retardation. This proposition of less polar transition state in the reaction mechanism is also supported by high negative entropy of activation which suggests a cyclic transition state where the charge is more dispersed. Further, if the change in rate constants with change in solvent polarity is only due to the stability of reactants or transition state, then the rate constant should also increase with decrease in polarity for the nonpolar and less polar solvents. However, the opposite trend in this case proposes the existence of reverse micellar aggregation of CTADC in non-polar solvents. Quaternary ammonium and phosphonium salts, exist as ion pairs or an aggregation of ion pairs in nonaqueous medium.[79] The degree of aggregation of these salts in a non-polar solvent is inversely proportional to the polarity of the medium.[80] With increase in non-polarity of the solvents, reverse micellization increases which results in higher partition of oxidant and substrate into two different phases imparting a rate decrease.

The above analysis may lead to the following proposition; (i) for the polar group of solvents, polarity (or hydrophilicity) is predominant due to solvation of reactants and transition state through polar-polar specific and nonspecific solute solvent interactions; and (ii) for the second group of solvents, non-polarity or hydrophobicity is predominant due to the formation of reverse micellar aggregates.

To confirm this proposition of differential contribution, the reactivity was correlated with log *P* (the logarithm of partition coefficient of the substrate between octanol and water). It has the inherent characteristics of both hydrophilicity (partition from or to water) and hydrophobicity (partition from or to octanol).[81] The bilinear plot of k_{obs} versus log *P* of the solvents (Figure 4.13), indicates balanced contribution from both the factors. A maximum at ethylacetate (log *P* = 0.73) in the plot indicates a hydrophobic switch, which balances the polarity and hydrophobic contributions of the solvents. In the lower side of log *P* values (<0.82), increasing hydrophobicity has a rate acceleration, whereas with higher log P values (> 0.73) rate retardation is observed. Solvents with log *P* values less than 0.73 solvate the reactants and the transition state through polar interactions (hydrophilicity) and the solvents with log P values more than 0.82 solvate through apolar interactions (hydrophobicity), whereas the common solvents ethylacetate and THF solvate through both polar and apolar interactions. This type of polarity switch (at log *P* = 0.88) has also been reported in the literature in the study of solvation of *p*-nitroaniline in alcohol-dioxane binary solvent mixtures and attributed the observation to the differential contribution of hydrophilic and hydrophobic parts of the solvents leading to preferential solvation.[82] Panigrahi *et al*.[83] have reported similar reversal in solvatochromism of some hydrophobic styrylpyridinium dyes and explained the fact through structural transition of the solvation shell with change in nature and polarity of the solvents.

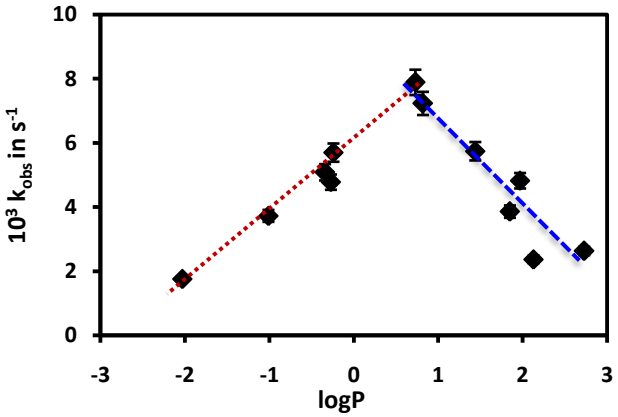


Figure 4.13 Plot of 10^3 x k_{obs} versus log *P* of the solvents in the oxidation reaction of isoniazid with CTADC at 298 K. (Dotted lines are drawn manually to show variations)

For the polar group of solvents, an increase in both anion solvating power (A) and cation solvating power (B) (Figure 4.14) of the solvents have adverse effect on the rate constant delineating less ionic and nonpolar transition state. These results also support the formation of cyclic transition state with charge delocalization in the rate-determining step. With increase in A and B, solvation of the complex increases through specific solvation of the respective ionic part by the solvents and thus the reactivity decreases.

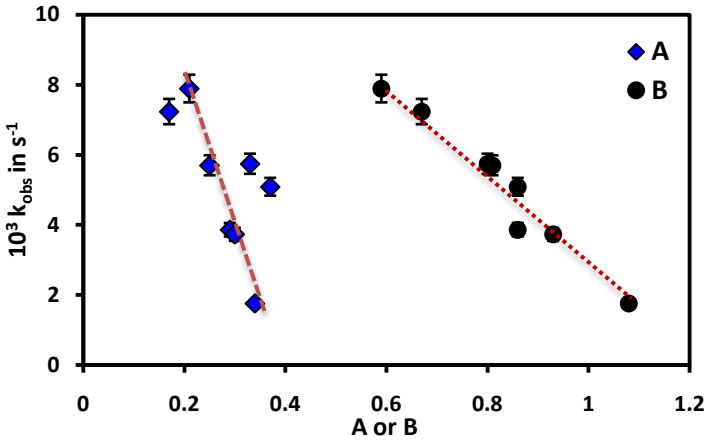
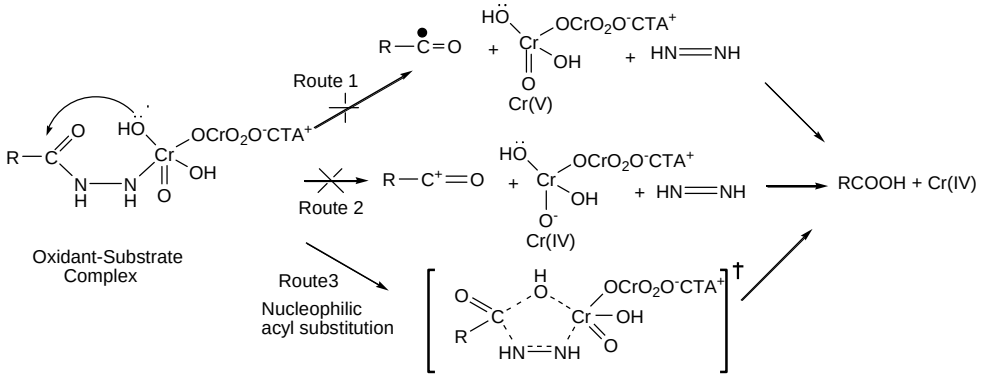


Figure 4.14 Plot of 10^3 x k_{obs} versus A and B of the solvents in the oxidation reaction of isoniazid with CTADC at 298 K.

Similar to the mechanism described by Amos *et. al*.[45] for the oxidation of isoniazid, other mechanisms may also be proposed as in Scheme 4.5. Possibility of route 1 leading to isonicotinoyl radical is ruled out due to the reasons mentioned earlier. Route 2, shows the cleavage of C-N bond to form free isonicotinoyl cation, diazene and Cr(VI). However, the results from effect of solvents and effect of temperature on rate constants precluded route 2. Thus, it may be proposed that in nonpolar environment, the breaking of C-N bond to form isonicotinoyl cation and attack of chromate hydroxyl group to the electrophilic isonicotinoyl cation are simultaneous leading to a nonpolar cyclic transition state, solvated and stabilized by nonpolar medium (route 3). Any change in the functionality of the oxidant-substrate complex which increases the nucleophilicity of the hydroxyl group, electrophilicity of acyl carbon and hydrophobicity of the reaction center leads to increase in the rate of nucleophilic acyl substitution through route 3.



(Scheme 4.5)

Our results may assist in understanding why a single Ser315Thr mutation in the KatG enzyme in Mycobacterium tuberculosis results in an enzyme without the ability to activate isoniazid.[24,84] The change from a serine with a primary alcoholic side chain to a threonine with secondary hydroxyl group in the substrate access channel of heme active site,[23-24,85] increases the nucleophilicity of oxygen functionality and also hydrophobicity in the active site. This change may favor the simultaneous acyl

nucleophilic substitution in preference to radical mechanism and therefore, the altered catalase-peroxidase confers resistance to isoniazid.

4.4 CONCLUSION

In conclusion, the oxidant (CTADC) oxidizes isoniazid to isonicotinic acid following Michaelis-Menten kinetics, the first step of which is a very fast formation of association complex between isoniazid and CTADC, followed by a second rate-determining dissociation of the complex proceeding via a less ionic and less polar transition state. CTADC assembles to form reverse micelle in non-polar solvents with cations at the interface providing different residing sides for the reactants thus, mimicking enzyme activity. The substrate and oxidant are partitioned into two different types of environments and, accordingly the reaction is controlled. The outcome from solvent effect illustrates the presence of reversal in "solvatoreactivity" with a polarity/hydrophobic switch (at log P = 0.73), due to differential contribution from nonpolar and polar solvents. Nonpolar solvents contribute more to the reverse micellization whereas polar solvents contribute to the stability of reactants and transition state. CTADC provides Cr(VI) for oxidation of substrate as well as it provides reverse micellar aggregates resembling that of lipid cell membrane. In this hydrophobic environment isoniazid is oxidized to isonicotinic acid. As a result of these investigations we speculate that the resistance conferred by the Ser315Thr mutant KatG is because of the preference of acyl nucleophilic substitution over the formation of acyl radical in hydrophobic environment, hindering the formation of INH-NAD adduct (**3**). An understanding of the mechanism of drug resistance may provide new directions in the development of more effective antibiotics for MDR-TB.

4.5 REFERENCES

1. Valadas, E.; Antunes, F. *Eur. J. Radiol.* ***2005***, *55*, 154.
2. Dye, C.; Williams, B. G. *Science* **2010**, *328*, 856.
3. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.
4. Liu, Y. X.; Xiao, C. L.; Wang, Y. X.; Li, Y. H.; Yang, Y. H.; Li, Y. B.; Bi, C. W.; Gao, L. M.; Jiang, J. D.; Song, D. Q. *Eur. J. Med. Chem*. **2012**, *52*, 151.
5. Bardou, F.; Quemard, A.; Dupont, M. A.; Horn, C.; Marchal, G.; Daffe, M. *Antimicrob. Agents Chemother.* **1996**, *40*, 2459.
6. Blanchard, J. S. *Annu. Rev. Biochem*. **1996**, *65*, 215.
7. Stoeckle, M. Y.; Guan, L.; Riegler, N.; Weitzman, I.; Kreiswirth, B.; Kornblum, J.; Laraque, F.; Riley, L. W. *J. Infect. Dis.* **1993**, *168*, 1063.
8. Heym, B.; Alzari, P. M.; Honore, N.; Cole, S. *Mol. Microbiol.* **1995**, *15*, 235.
9. Bloom, B. R.; Murray, C. J. L. *Science* **1992**, *257*, 1055.
10. Janin, Y. L. *Bioorg. Med. Chem.***2007**, *15*, 2479.
11. Wang, Y. X.; Yang, P.; Zhang, H.; Kong, W. J.; Li, Y. H.; Liu, F.; Gao, R. M.; Liu, T.; Jiang, J. .D.; Song, D. Q. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6004.
12. Yang, P.; Song, D. Q.; Li, Y. H.; Kong, W. J.; Wang, Y. X.; Gao, L. M.; Liu, S. Y.; Cao, R. Q.; Jiang, J. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 675.
13. Li, Y. H.; Yang, P.; Kong, W. J.; Wang, Y. X.; Hu, C. Q.; Zuo, Z. Y.; Wang, Y. M.; Gao, H.; Gao, L. M.; Feng, Y. C.; Du, N. N.; Liu, Y.; Song, D. Q.; Jiang, J. D. *J. Med. Chem.* **2009**, *52*, 492.
14. Wang, Y. X.; Wang, L.; Xu, Y. N.; Li, Y. H.; Jiang, J. D.; Si, S. Y.; Li, Y. B.; Ren, G.; Shan, Y. Q.; Hong, B.; Song, D. Q. *Eur. J. Med. Chem.* **2011**, *46*, 1066.
15. Ramani, A. V.; Monika, A.; Indira, V. L.; Karyavardhi, G.; Venkatesh, J.; Jeankumar, V. U.; Manjashetty, T. H.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2764.
16. Sinha, B. K. *J. Biol. Chem.* 1983, **258**, 796.
17. Zhang, Y.; Heym, B.; Allen B.; Young, D.; Cole, S. *Nature* **1992**, *358*, 591.
18. Johnsson, K.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 7425.
19. Johnsson, K.; King, D. S.; Schultz, P. G. *J. Am. Chem. Soc.* **1995**, *117*, 5009.
20. Wengenack, N.; Hoard, H. M.; Rusnak, F. *J. Am. Chem. Soc.* **1999**, *121*, 9748.
21. Wengenack, N.; Rusnak, F. *Biochemistry* **2001**, *40*, 8990.
22. Wengenack, N. L.; Lopes, H.; Kennedy, M. J.; Tavares, P.; Pereira, A. S.; Moura, I.; Moura, J. J. G.; Rusnak, F. *Biochemistry* **2000**, *39*, 11508.
23. Zhao, X.; Yu, H.; Yu, S.; Wang, F.; Sacchettini, J. C.; Magliozzo, R. S. *Biochemistry* **2006**, *45*, 4131.

24. Joanis, B. S.; Souchon, H.; Wilming, M.; Johnsson, K.; Alzari, P. M.; Cole, S. T. *Biochem. J.* **1999**, *338*, 753.

25. Lukat-Rodgers, G. S.; Wengenack, N. L.; Rusnak, F.; Rodgers, K. R. *Biochemistry* **2001**, *40*, 7149.

26. Ghiladi, R. A.; Medzihradszky, K. F.; Rusnak, F. M.; deMontellano, P. R. O. *J. Am. Chem. Soc.* **2005**, *127*, 13428.

27. Delaine, T.; Genisson, V. B.; Meunier, B.; Bernadou,J. *J. Org. Chem.* **2007**, *72*, 675.

28. Amos, R. I. J.; Schiesser, C. H.; Smith, J. A.; Yates, B. F. *J. Org. Chem.* **2009**, *74*, 5707.

29. Broussy, S.; Genisson, V. B.; Quemard, A.; Meunier, B.; Bernadou,J. *J. Org. Chem.* **2005**, *70*, 10502.

30. Rozwarski, D. A.; Grant, G. A.; Barton, D. H. R.; Jacobs, W. R.; Sacchettini, J. C. *Science* **1998**, *279*, 98.

31. Wilming, M.; Johnsson, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 2588.

32. Nguyen, M.; Claparols, C.; Bernadou, J.; Meunier, B. *Chem Biochem*. **2001**, *2*, 877.

33. Ducasse-Cabanot S.; Cohen-Gonsaud, M.; Marrakchi, H.; Nguyen, M.; Zerbib, Bernadou, D. J.; Daffe, M.; Labesse G. Quemard, A. *Antimicrob. Agents Chemother*. **2004**, *48*, 242.

34. Banerjee, A.; Dubnau, E.; Quemard, A.; Balasubramanian, V.; Sun, U. K.; Wilson, T.; Collins, D.; de Lisle, G.; Jacobs, W. R. *Science* **1994**,*263*, 227.

35. Takayama, K.; Wang, L.; David, H. L. *Antimicrob. Agents Chemother*. **1972**, *2*, 29.

36. Quemard, A.; Lacave, C.; Laneelle, G. *Antimicrob. Agents Chemother*. **1991**, *35*, 1035.

37. Braslau, R.; Anderson, M. O.; Rivera, F.; Jimenez, A.; Haddad, T.; Axon, J. R. *Tetrahedron*, **2002**, *58*, 5513.

38. Rickman, K. A.; Swancutt, K. L.; Mezyk, S. P.; Kiddle, J. *J. Bioorga. Med. Chem. Lett.* **2013**, *23*, 3096.

39. Yalgudre, R. S.; Gokavi, G. S. *Ind. Eng. Chem. Res*. **2012**, *51*, 5135.

40. Magliozzo, R. S.; Marcinkeviciene, J. A. *J. Biol. Chem.* **1997**, *272*, 8867.

41. Shoeb, H.A.; Bowman, B. U.; Ottolenghi, A.C.; Merola, A. *J. Antimicrob. Agents Chemother*. **1985**, *27*, 399.

42. Puttaswamy; Anuradha, T. M.; Ramachandrappa, R.; Gowda, N. M.M. *Int. J. Chem. Kinet*. **2000**, *32*, 221.

43. Kulkarni, S. D.; Nandibewoor, S.T. *Trans. Metal Chem.* **2006**, *31*, 1034.

44. Kulkarni, R. M.; Bilehal, D. C.; Nandibewoor, S. T. *Analyt. Sci.* **2004**, *20*, 743.

45. Amos, R. I. J.; Gourlay, B. S.; Schiesser, C. H.; Smith, J. A.; Yates, B. F. *Chem. Comm.* **2008**, 1695.

46. Angel-Yagui, C. O.; Pessoa-Jr, A.; Tavares, L. C. *J. Pharm. Pharmaceut. Sci.* **2005**, *8*, 147.
47. Patel, S.; Mishra, B. K. *Tetrahedron*, **2007**, *63*, 4367.
48. Mukherjee, S.; Chakraborty, M.; Panda, A. K.; Bhattacharya, S.C.; Moulik, S.P. *Colloids Surf. A: Physicochem. Eng. Asp.* **2011**, *388*, 1.
49. Patel, S.; Mishra, B. K. *Tetrahedron Lett.* **2004**, *45*, 1371.
50. Sahu, S.; Patel, S.; Mishra, B. K. *Synth. Commun*. **2005**, *35*, 3123.
51. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006**, *71*, 352.
52. Vogel, A. I. In A Textbook of Quantitative Inorganic Analysis, 3rd ed.; ELBS and Longmans: U.K., 1961.
53. Riddick, J. A.; Bunger, W. B. Organic Solvents; Techniques of Chemistry, Vol. II, Wiley-Interscience: New York, 1970.
54. Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochmann, M. In Advanced Inorganic Chemistry, 6th ed.; John Wiley & Sons: New York, 1999; p 747.
55. Khan, A.A.P.; Asiri, A.M.; Azum, N.; Rub, M.A.; Khan, A.; Al-Youbi, A.O. *Ind. Eng. Chem. Res.* **2012**, *51*, 4819.
56. Hegde, R.N.; Shetti, N.P.; Nandibewoor, S.T. *Ind. Eng. Chem. Res.* **2009**, *48*, 7025.
57. Laidler, K. J. In Chemical Kinetics, 2nd ed.; McGraw-Hill: New York, 1968; p 327.
58. Patel, S.; Mishra, B. K. *Int. J. Chem. Kinet.* **2006**, *38*, 651.
59. Patel, S.; Mishra, B. K. *J. Org. Chem.* **2006,** *71*, 6759.
60. Yalgudre, R.S.; Gokavi, G.S. *Ind. Eng. Chem. Res.* **2012**, *51*, 5135.
61. Bodiguel, J.; Nagy, J M.; Brown, K. A.; Jamart-Gregoire, B. *J. Am. Chem. Soc.* **2001**, *123*, 3832.
62. Karimi, M.A.; Mehrjardi, A. H.; Ardakani, M. M.; Ardakani, R. B.; Mashhadizadeh, M.H.; Sargazi, S. *Int. J. Electrochem.* Sc. **2010**, *5*, 1634.
63. Wiberg, K. B. *Chem. Rev.* **1955**, *55*, 713.
64. Deepalakshmi, S.; Sivalingam, A.; Kannadasan, T.; Subramaniam, P.; Sivakumar, P.; Brahadeesh, S.T. *Spectrochim. Acta*. A **2014**, *124*, 315.
65. Collins, C. J.; Bowman, N. S. Isotope Effects in Chemical Reactions; van Nostrand-Reinhold: New York, 1970; p 267.
66. Kuotsu, B.; Tiewsoh, E.; Debroy, A.; Mahanti, M. K. *J. Org. Chem.* **1996**, *61*, 8875.
67. Khan, A.A.P.; Mohd, A.; Bano, S.; Siddiqi, K.S. *Ind. Eng. Chem. Res.* **2011**, *50*, 9883.
68. El-sharkawy, R. G.; Badr El-din, A. S.; Etaiw, S. El-din H. *Spectrochim. Acta. A*, **2011**, *79*, 1969.
69. Correa, N. M.; Silber, J. J.; Riter, R. E.; Levinger N. E. *Chem. Rev.* **2012**, *112*, 4569.

70. Rosso, F. D.; Bartoletti, A.; Profio, D. P.; Germani, R.; Savelli, G.;Blasko, A.; Bunton, C. A. *J. Chem. Soc. Perkin. Trans.* **1995**, *2*, 673.

71. Fletcher, P. D. I.; Galal, M. F.; Robinson, B. H. *J. Chem. Soc. Faraday Trans.1* **1985**, *81*, 2053.

72. Tessy, E. I.; Rakshit, A. K. *Bull Chem. Soc. Jpn.* **1995**, *68*, 2137.

73. Jimenez-Carmona, M. M.; Luque de Castro, M. D. *Anal. Chem.* **1998**, *70*, 2100.

74. Majhi, P. R.; Blume, A. *J. Phy. Chem. B* **2002**, *106*, 1075.

75. Yue, H.; Guo, P.; Guo, R. *J. Chem. Eng. Data* **2009**, *54*, 2923.

76. Tah, B.; Pal, P.; Mahato, M.; Talapatra, G. B. *J. Phys. Chem.B* **2011**, *115*, 8493.

77. Chakraborty, H.; Sarkar, M. *Langmuir* **2004**, *20*, 3551.

78. Cui, X.; Jiang, Y.; Yang, C.; Lu, X.; Chen, H.; Mao, S.; Liu, M.; Yuan, H.; Luo, P.; Du, Y. *J. Phys. Chem. B* **2010**, *114* , 7808.

79. Brandstrom, A. *Adv. Phys. Org. Chem*. **1977**, *15*, 267.

80. Starks, C. M.; Owens, R. M. *J. Am. Chem. Soc.* **1973**, *95*, 3616.

81. Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.

82. Patel, S.; Gorai, S.; Malik P.K. *J. Photochem. Photobiol. A* **2011**, *219*, 76.

83. Panigrahi, M.; Dash, S.; Patel, S.; Behera, P. K.; Mishra, B. K. *Spectrochim. Acta. A* **2007**, *68*, 757.

84. Rouse, D.A.; DeVito, J.A.; Li, Z.; Byer, H.; Morris, S.L. *Mol. Microbiol.* **1996**, *22*, 583.

85. Cade, C.E.; Dlouhy, A.C.; Medzihradszky, K.F.; Salas-Castillo, S.P.; Ghiladi, R.A.; *Protein Sci.* **2010**, *19*, 458.

Chapter 5

Oxidation of Carbamazepine by Cetyltrimethylammonium Permanganate

5.1 INTRODUCTION

Carbamazepine (CBZ) is an active pharmaceutical ingredient, chemically known as 5*H*-dibenzo[*b,f*]azepine-5-carboxamide. It is widely used as anticonvulsant, antiepileptic, and mood-stabilizing drug.[1-3] It is also used in the treatment of other diseases such as resistant schizophrenia, ethanol withdrawal, neuropathic pain, restless leg syndrome, psychotic behaviour associated with dementia, and post-traumatic stress disorders.[4-7] Although CBZ is one of the most widely used drug all over the world, its use is associated with a range of adverse reactions mainly skin rash, blood disorders, hepatitis, and hematological disorders.[8-14] Albeit the mechanism of carbamazepine-induced adverse reactions is not clear, it is believed to be due to the formation of different chemically reactive oxidative metabolites and their subsequent covalent binding to cellular proteins.[15-18] Thus a brief idea about CBZ oxidative metabolism in diverse environment is desirable to get invaluable information on the mechanistic pathway, cause, and the condition for oxidative instability. The most important pathway in CBZ metabolism is the cytochrome P450 (CYP3A4) catalyzed formation of carbamazepine-10,11-epoxide (CBZ-EP), which is further hydrolyzed to 10,11-dihydro-10,11-dihydroxy carbamazepine (CBZ-DiOH) and under suitable conditions oxidized to dicarboyl products.[19,20] Various biomimetic models are developed to mimic the function of cytochrome P450 to provide information regarding production of drug metabolites and to understand the mechanism of biological transformation in CBZ oxidation. Some examples include Fe(II) and Mn(II) complexes of rigid crossed- bridged tetraazamacrocycles along with oxidants H_2O_2 and $KHSO_{5,}$[21] metalloporphyrin complexes along with a variety of oxidants such as ClO^-, HSO_5^-, *m*-chloroperoxybenzoic acid, H_2O_2, [22-24] and Jacobsen catalyst.[25]

In case of the major human CYP isoform, that is, CYP3A4, the major interaction with substrates arises from hydrophobic interaction as evident from structure activity relationship (SAR) studies and available data on CYP3A4 crystal structure.[26-28] The large active site of CYP3A4 is unique among CYP isozymes

containing seven phenylalanine residues stacking against each other forming a hydrophobic core.[26-28]

Objectives: Owing to the importance of hydrophobic forces in the substrate binding in the cytochrome-mediated oxidative metabolism in CBZ oxidation, and selectivity of cetyltrimethylammonium permanganate CTAP toward the oxidation of olefinic double bonds in nonpolar medium,[29] herein we have made an attempt to look into the mechanism of oxidative metabolism of CBZ by CTAP in a surfactant-generated hydrophobic and biomimetic environment and to answer the following pertinent questions.

- Will CTAP act as a selective oxidant for carbamazepine ?
- If so, what may be the reason of selectivity, hydrophobicity associated with CTA group or the selectivity of permanganate for double bonds?
- What will be the mechanism of the oxidation reaction?

To achieve the above objectives and to answer the above hypothesis, oxidation products have been analyzed, reaction kinetics has been studied studied and a suitable mechanism for the reaction has been proposed.

5.2 EXPERIMENTAL

5.2.1 Materials

CBZ was purchased from Sigma-Aldrich (Mumbai, India) and used without further purification. The organic solvents were purified by standard methods. The surfactants CTAB and SDS (Merck, India) were purified by recrystallization from aqueous ethanol. TX-100 was purchased from Merck (India) and used without further purification.

5.2.2 Synthesis of cetyltrimethylammonium permanganate (CTAP)

Cetyltrimethylammonium permanganate (CTAP) was prepared as reported earlier[29] by adding aqueous solution of potassium permanganate (2 g, 0.06M) the aqueous solution of cetyltrimethylammonium bromide (0.84 g, 0.01M) with

continuous stirring using a teflon coated magnetic bar at room temperature. A violet coloured compound appeared immediately. Stirring was continued for 15 minutes more after completion of the addition of the permanganate solution. The resulting violet product was then filtered off and washed with water several times till no bromide and permanganate were detected in the filtrate. It was vacuum dried and kept in a desiccator in the dark. M.P. 225 ^{0}C (decomposed), Yield: 98%, and its purity was checked from its NMR spectral data.

5.2.3 Kinetic Measurements

The kinetics of oxidation of CBZ by CTAP was studied spectrophotometrically using a double beam Shimadzu UV-vis spectrophotometer (UV-1800). The temperature in the reaction cell was controlled by circulating water by using Lauda thermostat within a temperature fluctuation of 0.05^{o}C. CTAP stock solution was prepared in acetonitrile because of considerable stability in this solvent. The reactions were performed under pseudo-first-order conditions by keeping excess of the CBZ (10 times or more) with respect to CTAP. The rate of disappearance of the Mn(VII) species was followed by monitoring the depletion of absorption band at 530 nm. The first-order rate constants k_{obs} were obtained from the linear plot of log [CTAP] against time for up to 75% completion of the reaction. The values reported are the average of at least triplicate runs and were reproducible within ±6% error.

5.2.4 Stoichiometry

The stoichiometry of the reaction was determined by performing the experiment at 298 K, under the condition of [CTAP] > [CBZ] at varying carbamazepine concentration. The disappearance of Mn(VII) was followed, until the absorbance values become constant. The CTAP was estimated after 7 h. The stoichiometric ratio was found to be 2:1 CTAP to CBZ, respectively.

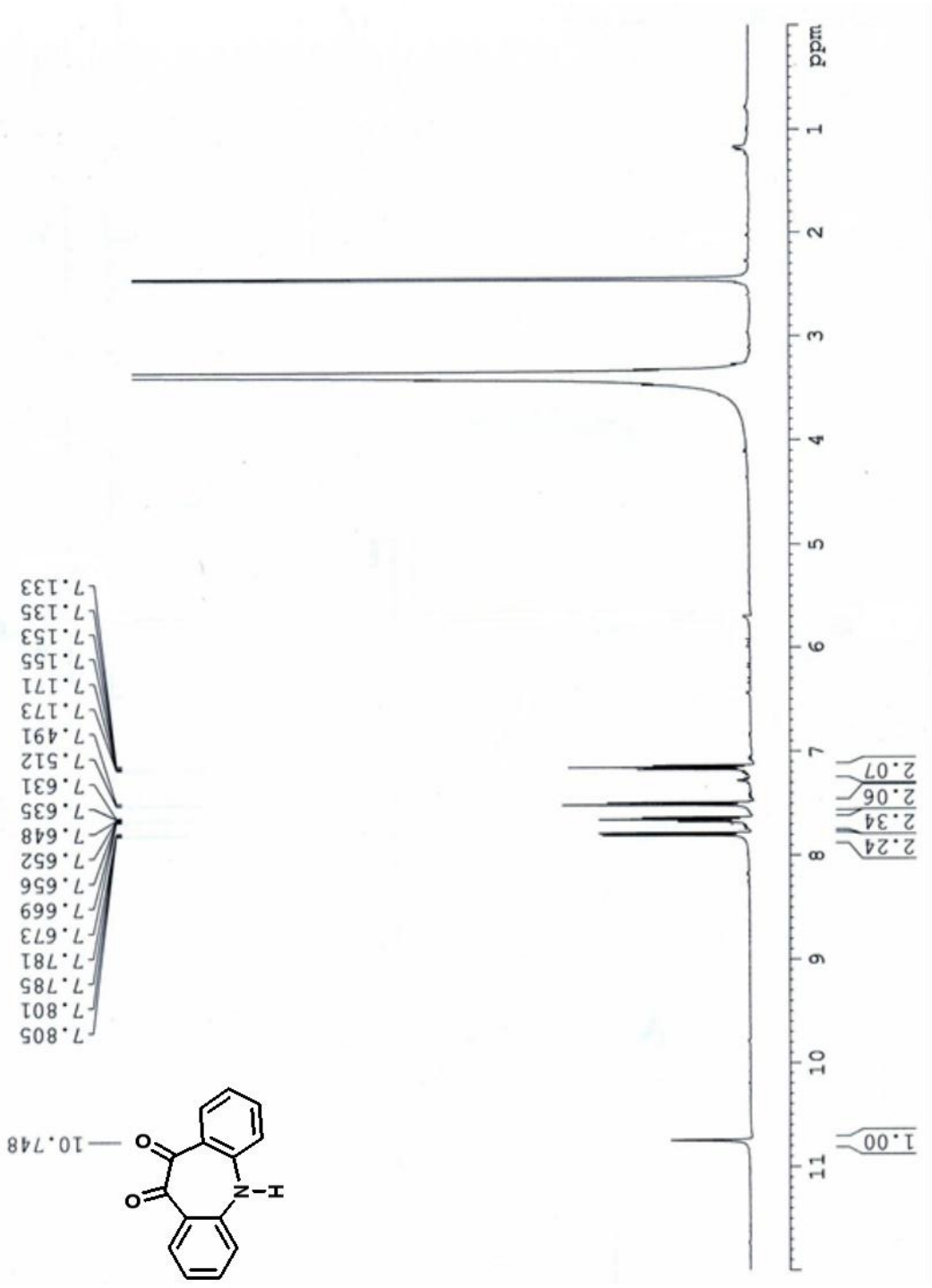


Figure 5.1 ^{1}H-NMR spectrum of 1*H*-dibenzo[*b,f*] azepine-4,5-dione.

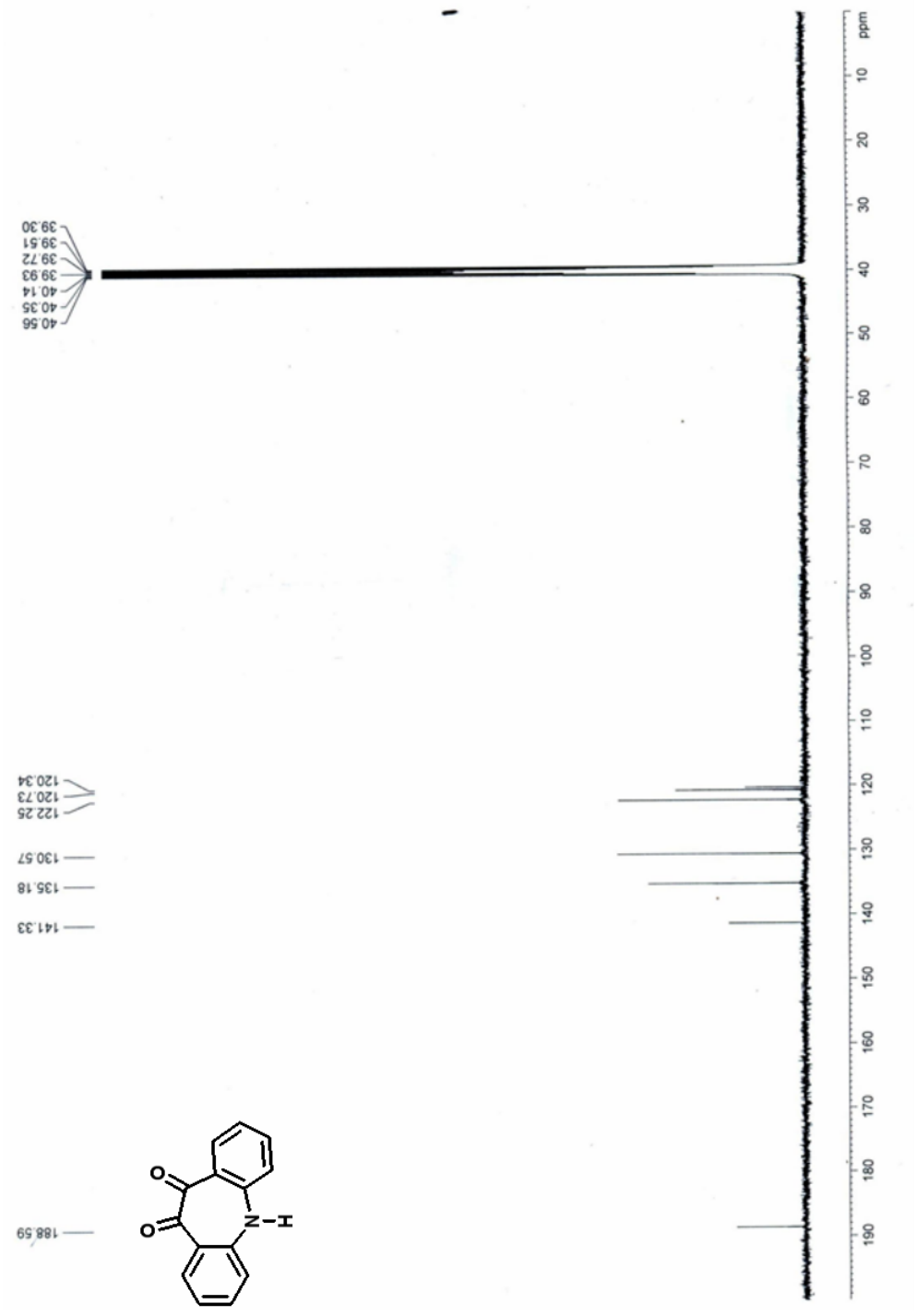


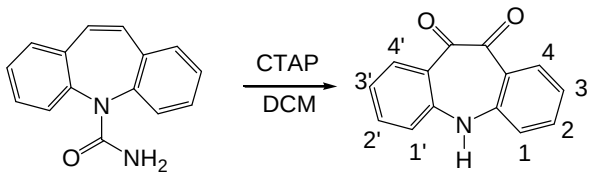
Figure 5.2 ^{13}C-NMR spectrum of 1*H*-dibenzo[*b,f*] azepine-4,5-dione.

5.2.5 Product analysis

The reaction mixture containing CTAP (160 mg, 1M) and CBZ (94 mg, 1M) in DCM was stirred and refluxed for 30 minutes. The completion of the reaction was monitored by TLC. The solvent was evaporated, and the reaction mixture was subjected to column chromatography by taking hexane-ethylacetate (8:2) mixture as an eluent. The orange coloured solid product thus obtained (48 mg, yield 51%, M. Pt. >300°C) was characterized from ^{1}H NMR, ^{13}C NMR, and mass spectral analysis. ^{1}H NMR (400 MHz, DMSO-*d6*): δ/ppm 10.748 (1H, S) for the -NH proton, 7.8 (2H,q) for 4,4$^{'}$ proton, 7.67 (2H, m) for 2,2$^{'}$ proton, 7.5 (2H, d) for 3,3$^{'}$ proton, 7.17 (2H, m) for 1,1$^{'}$proton (Figure 5.1). ^{13}C NMR (100 MHz, DMSO-d6): δ/ppm 120.34, 120.73, 122.25, 130.57, 135.18, 141.33, 188.59 (Figure 5.2). MS (ESI, positive mode): m/z (relative intensity) 246 ([M + Na]$^{+}$, 100), 469 ([2M + Na]$^{+}$, 80) MS (ESI, negative mode): m/z (relative intensity) 222 ([M – H]$^{+}$, 100). NMR and Mass spectral analysis of the product confirmed the formation of 1*H*-dibenzo[*b,f*] azepine-4,5-dione.

5.3 RESULTS AND DISCUSSION

The reaction mixture consisting of CTAP and CBZ in DCM was stirred and refluxed for 30 minute. The completion of the reaction was monitored using TLC. The mixture turned brown, indicating the formation of reduced Mn(IV). The product was obtained from the reaction mixture as an orange solid, which was characterized by its NMR and Mass spectral data as 1*H*-dibenzo[*b, f*]azepine-4,5-dione (**1**) (Scheme 5.1).



1H-dibenzo[b,f] azepine-4,5-dione

1

(Scheme 5.1)

From the stoichiometric analysis, it has been found that 1 mole equiv of CBZ reacts with 2 mole equiv of CTAP. A free radical pathway may be ruled out for the present reaction as (i) oxidation of CBZ by CTAP, in an atmosphere of nitrogen, failed to induce polymerization of acrylonitrile[30] and (ii) the rate of the reaction was unaffected by the addition of acrylonitrile.[31] Thus, Mn(VII) may be reduced to Mn(III) and Mn(V) and further changed to Mn(IV) by a disproportionation reaction in a sequential manner (Scheme 5.2). The formation of Mn(IV) in the oxidation of various organic substrates by Mn(VII) is also well known in the literature.[32]

2 Mn(VII) + Carbamazepine ⟶ 1*H*-dibenzo[*b,f*]-azepine-4,5-dione + Mn(III) + Mn(V)

Mn(III) + Mn(V) ⟶ 2 Mn(IV)

Hence, 2Mn(VII) ⟶ 2 Mn(IV)

(Scheme 5.2)

In the oxidative transformation of CBZ, using Mn(VII) as an oxidizing system, Hu *et al.* detected 12 different intermediates and products, whereas using Fe(VI) as an oxidizing system they have detected 6 products.[33] They proposed that reactions are initiated by electrophilic attack at the olefinic double bond on the central heterocyclic ring by Mn(VII) or Fe(VI). Rao *et al*. have detected 14 intermediates and products by using WO_3 suspension and proposed an electrophilic attack of radicals at the olefinic double bond in the central heterocyclic ring, leading to a ring-opening and formation of various products.[34] In the present oxidizing system, a single product was detected and isolated in neutral medium, indicating the mildness and chemoselectivity of the oxidizing system. Further, similar to biological system, it is oxidized at the double bond to form the dicarbonyl product which indicates the biomimetic characteristics of the oxidizing system. For the mechanistic analysis of this selective oxidative transformation, the rates of reaction were monitored by observing the rate of depletion of Mn(VII) at 530 nm under pseudo-first order condition keeping [CBZ] more than 10-fold excess relative to the [oxidant]. The observed rate constants (k_{obs}) were calculated from the linear plots of log [CTAP] versus time and presented in

Table 5.1. Rates of the reaction at various concentrations of the oxidant and substrate were also calculated by multiplying [oxidant] with that of the corresponding k_{obs}.

5.3.1 Effect of reactant concentration

The observed rate constant was found to increase with an increase in [CBZ], whereas it was found to be almost constant with the change in the [CTAP]. The log (rate) values obtained at different conditions (Table 5.1) were subjected to multiple regression analysis and were correlated with the parameters of the reaction condition, i.e. [substrate] and [oxidant], to examine the interdependency of reactant concentrations towards the rate of the reaction. The regression model, thus obtained, is presented in equation 1. The linear plot (R^2 = 0.997) of observed rate versus predicted rate (Figure 5.3) supports the regression model. Using equation 1, the rate expression can be written as in equation 2. From the rate expression, it may be predicted that, the order of the reaction is 1 with respect to CTAP and 0.86 with respect to CBZ.

Log (rate) = -0.997(±0.070) + 0.984(±0.017) log [CTAP] + 0.856 (±0.023) log [Carbamazepine] (1)

R^2 = 0.998, F = 2977 n = 12

Rate= 0.1 $[CTAP]^{0.98}$ $[Carbamazepine]^{0.86}$ (2)

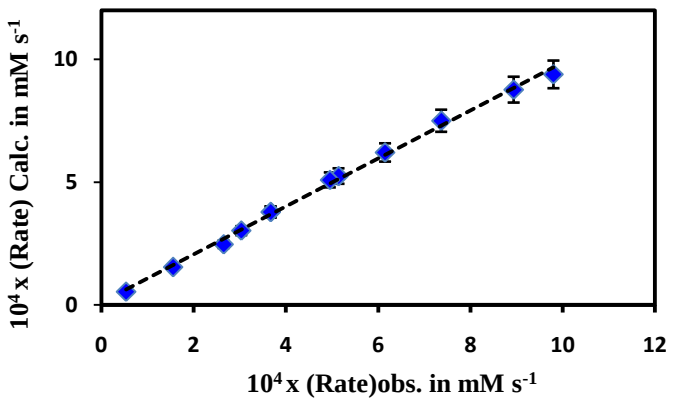


Figure 5.3 Plot of observed rate versus calculated rate of the reaction for the oxidation reaction of carbamazepine with CTAP in dichloromethane at 298 K.

Table 5.1 Effect of [carbamazepine] and [CTAP] on the oxidation of carbamazepine by CTAP in DCM at 298K

[CTAP] in mM	[Carbamazepine] in mM	10^4 x k_{obs} s^{-1}	10^4 x Rate (mM s^{-1})	
			Observed	Calculated[a]
0.047	4.5	11.46 ± 0.37	0.54	0.54
0.135	4.5	11.5 ± 0.51	1.55	1.54
0.269	4.5	11.28 ± 0.57	3.03	3.03
0.338	4.5	10.86 ± 0.38	3.67	3.79
0.471	4.5	10.92 ± 0.46	5.14	5.25
0.338	2.7	7.84 ± 0.25	2.65	2.47
0.338	4.5	10.86 ± 0.38[b]	3.67	3.79
0.338	6.4	14.66 ± 0.41	4.96	5.09
0.338	8.0	18.20 ± 0.30	6.15	6.21
0.338	10	21.80 ± 0.49	7.37	7.50
0.338	12	26.45 ± 0.78	8.94	8.77
0.338	13	29.00 ± 0.84	9.80	9.39

[a]Calculated using Eq (1)

[b]k_{obs} 10^4 at 293, 303, and 308 K are obtained to be 8.6 ± 0.25, 13.7 ± 0.42, and 17.24 ± 0.59 s^{-1} respectively.

The order of the reaction with respect to each reactant were also calculated from the slopes of linear plots of log (rate) versus log [reactant] using equation 3 and 4 and were found to be same.

$$\text{Rate} = k_2 [\text{CTAP}]^a [\text{Carbamazepine}]^b \quad (3)$$

$$\text{Log (rate)} = \log k_2 + a \log [\text{CTAP}] + b \log [\text{carbamazepine}] \quad (4)$$

where **a** and **b** are orders with respect to CTAP and carbamazepine.

The magnitude of **a** has been calculated from slope of the linear plot of log (rate) vs. log [CTAP] at constant concentration of carbamazepine (Figure 5.4). Similarly, magnitude of **b** can be calculated from the linear plot of log (rate) vs. log [carbamazepine] at constant CTAP concentration (Figure 5.5). The oxidation process is calculated to be first-order with respect to CTAP, while it is fractional order (0.84) with respect to carbamazepine.

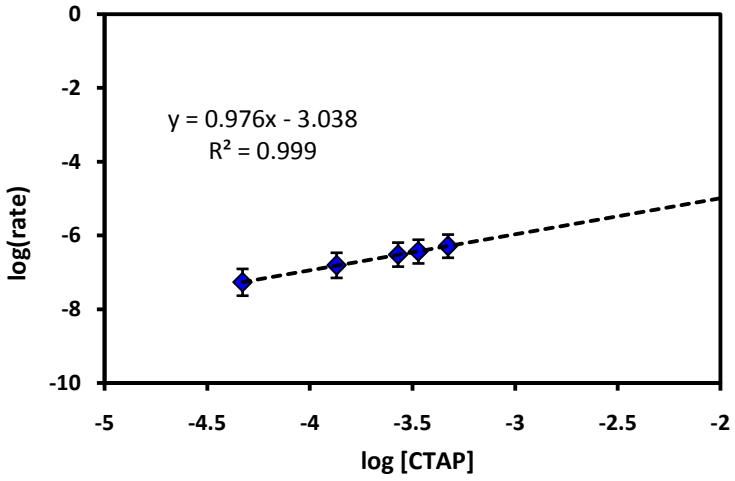


Figure 5.4 Plot of log (rate) versus log [CTAP] for the oxidation reaction of carbamazepine with CTAP in dichloromethane at 298 K.

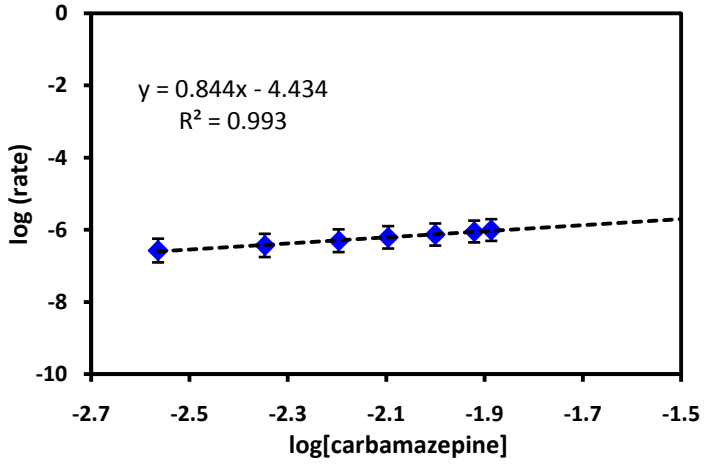


Figure 5.5 Plot of log (rate) versus log [carbamazepine] for the oxidation reaction of carbamazepine with CTAP in dichloromethane at 298 K.

Lee and Brownridge, [35] in the oxidation of cinnamic acid by permanganate ion, detected the hypomanganate ester intermediate, which appears yellow and exhibit

absorption maximum at 415 nm in the electronic spectrum. In the present case, the development of a broad shoulder at 400-430 nm in the UV-vis spectrum (Figure 5.6) during the reaction also indicates the formation of Mn(V)-ester intermediate, which decomposes to Mn(III) and the products.

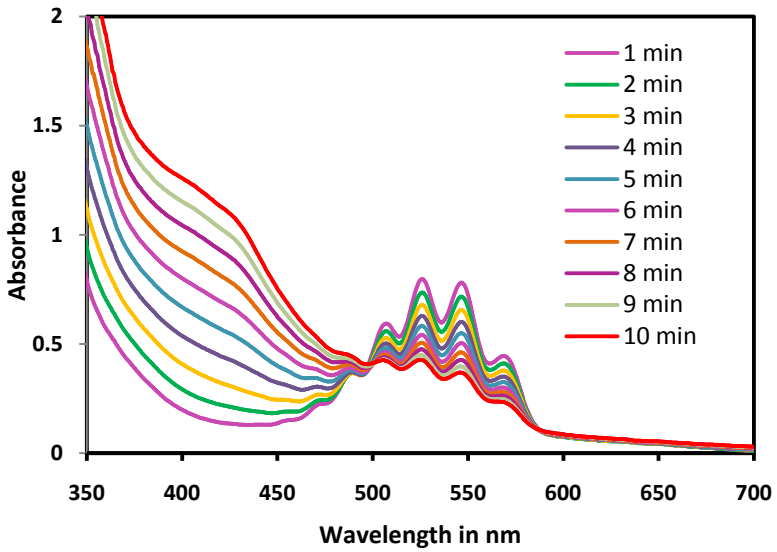
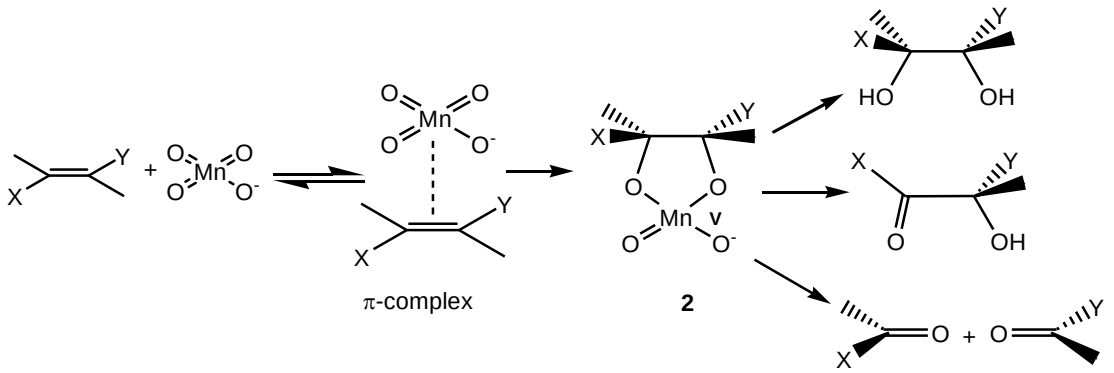


Figure 5.6 UV-vis spectra of reaction mixture consisting of CTAP and carbamazepine in DCM at different time interval.

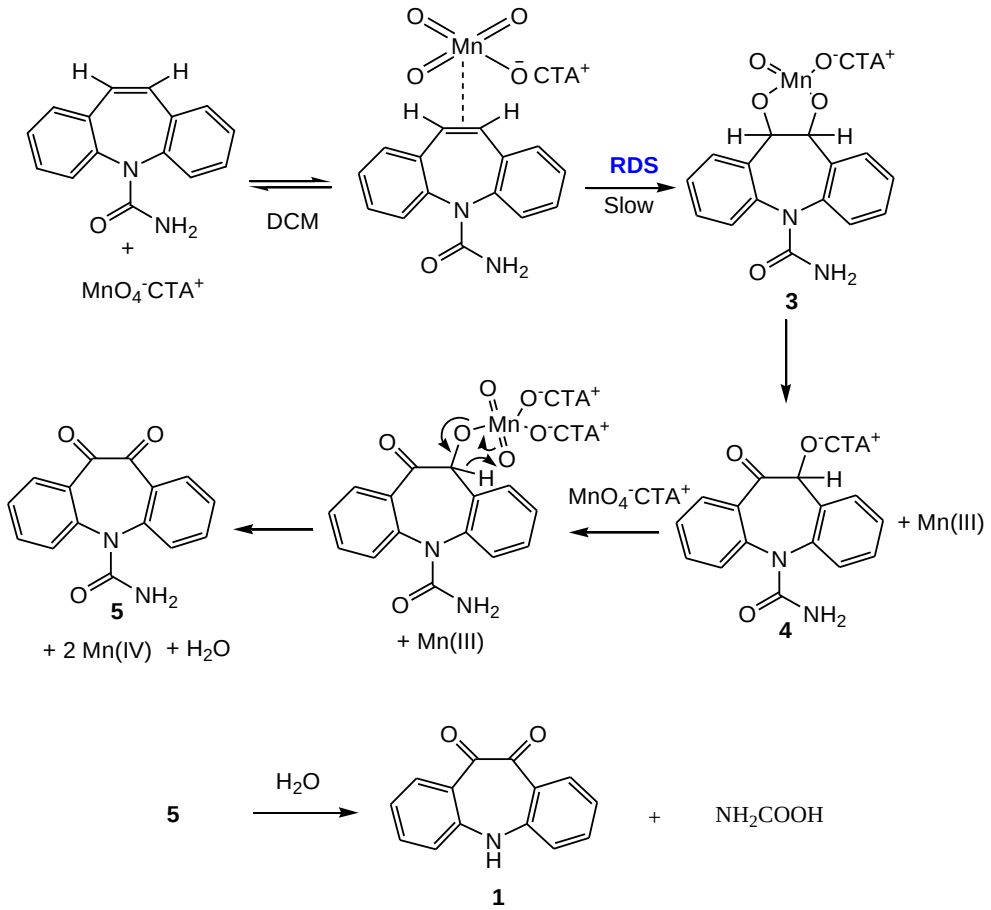
The permanganate ion oxidation of alkenes is proposed to proceed through an initial π-complex formation between electropositive metal and electron-rich alkene, followed by the slow rate-determining cycloaddition to form Mn(V)-ester intermediate (**2**).[36-37] The fate of the Mn(V)-ester intermediate is entirely dependent upon the reaction condition.[32] In acidic medium it cleaves to two carbonyl compounds while in basic condition it hydrolyses to diol. Furthermore, if the medium is neutral or slightly basic, it converts to α-hydroxycarbonyl compound (Scheme 5.3).[32, 38-40]

For the present oxidation reaction of CBZ by CTAP, a similar mechanistic path has been proposed (Scheme 5.4), where the first step involves a reversible formation of π-complex between CBZ and CTAP followed by a rate-determining *syn* addition of permanganate to the double bond of the CBZ, resulting in the formation of a cyclic hypomanganate ester intermediate (**3**). The possibilities of two paths, i.e. the hydrolysis of the intermediate to diol and cleavage of the intermediate to carbonyls

may be precluded for the present case as the medium is neither acidic nor basic and also devoid of water. Utilizing an additional permanganate ion, **3** may decompose to give the dicarbonyl product (**5**) and a molecule of water probably through an intermediate (**4**).[32, 38-40] The oxidation of **4** to **5** may go through removal of hydride ion with simultaneous reduction of Mn(VII) to Mn(V).[41] **5** then hydrolyses to give the final product **1** utilizing a molecule of water generated during the oxidation process.



(Scheme 5.3)



(Scheme 5.4)

5.3.2 Effect of solvent polarity

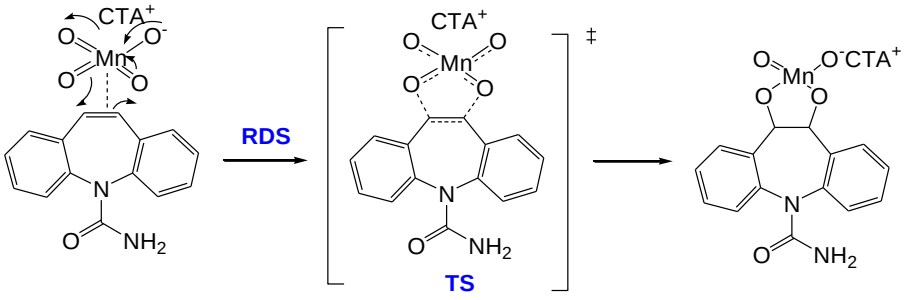
The effect of solvent on the reaction rate gives invaluable information regarding the reaction mechanism, nature of the transition states, and intermediates. The change in the rate with change in the nature of the solvents or the polarity of the solvents is mainly due to differential solvation of the starting material and transition state by the solvents. To see the effect of solvent polarity on the rate of reaction for the present investigation, eight different solvents were used having nonpolar and dipolar aprotic characteristics. The observed rate constants are tabulated in Table 5.2 and correlated with various solvent polarity parameters such as dielectric constant, π^*, dipole moment, anion-solvating power (A), cation-solvating power (B), and hydrophobicity (log P).

Table 5.2 Values of observed rate constants for the oxidation of carbamazepine by CTAP in different solvents at 298K. [CTAP] =0.338 mM [carbamazepine] = 4.5 mM

Solvents	**10^4 x k_{obs} in s^{-1}**
Toluene	36.24 ± 1.13
Benzene	32.62 ± 0.77
Chloroform	25.43 ± 1.13
Dichloromethane	10.86 ± 0.38
Acetonitrile	1.58 ± 0.07
Acetone	1.54 ± 0.05
Tetrahydrofuran	4.84 ± 0.16
Ethylacetate	11.46 ± 0.34

The plots of k_{obs} with A and B exhibit scattered points, indicating that no definite relationship exists between the ion-solvating power of the solvent and the reactivity. The plot of k_{obs} versus dielectric constant (Figure 5.6) shows an asymptotic decrease in the rate constant with an increase in polarity of the medium. There is a

sharp decrease in rate constant from 36.2 x 10^{-4} to 4.82 x 10^{-4} s^{-1} in the lower side of dielectric constant (<10), whereas in the higher side (10-40) it remains almost constant. A similar increasing trend with an increase in hydrophobicity is observed from the plot of k_{obs} versus log *P* of the solvents (Figure 5.7). These facts can be explained through the formation of a less polar cyclic transition state than the reactants and the differential solvation of reactants and the transition state. As shown in Scheme 5.5, the negative charge of the MnO_4^- is well dispersed in the transition state within the C=C and the permanganate moiety. In high polar solvents (with high dielectric constant), there is extensive solvation of the ionic reactant (MnO_4^-) leading to a decrease in the rate of attack of the permanganate toward CBZ. On the other hand, less polar solvents solvate the less polar transition state more extensively than the reactants leading to a higher rate of reaction.



(Scheme 5.5)

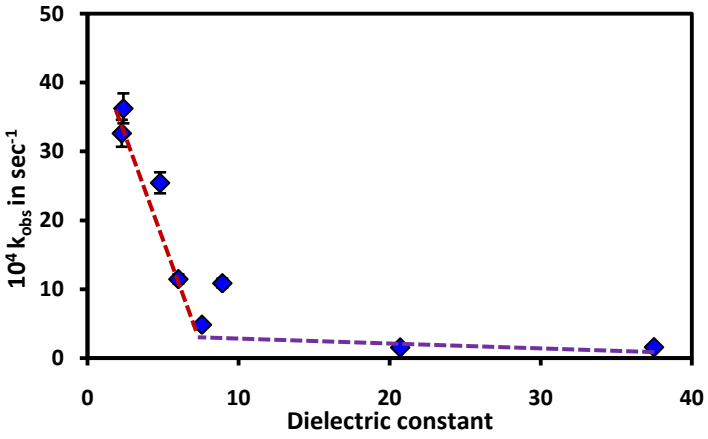


Figure 5.6 Plot of k_{obs} versus dielectric constant of the solvents in the oxidation reaction of carbamazepine by CTAP at 298 K. (The dotted lines are drawn manually to indicate the variations)

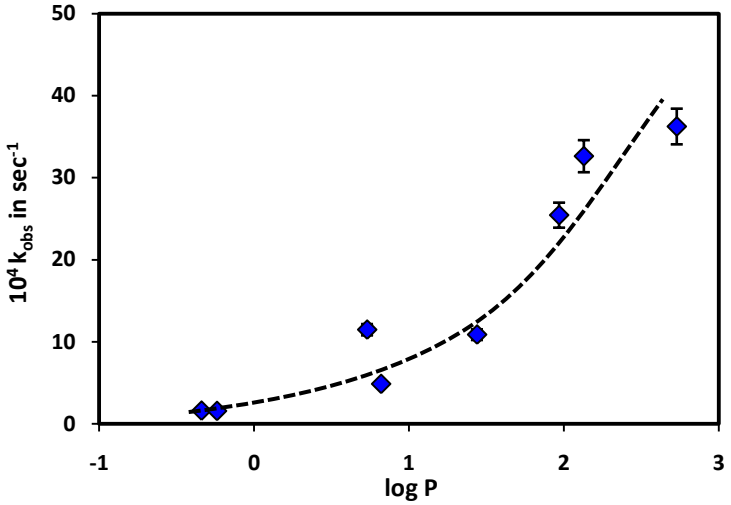


Figure 5.7 Plot of k_{obs} versus log P of the solvents in the oxidation reaction of carbamazepine by CTAP at 298 K. (The dotted lines are drawn manually to indicate the variations)

5.3.3 Effect of temperature

With an increase in temperature, the rate constant increases linearly (Table 5.1). Using Arrhenius and Eyring equation, thermodynamic parameters such as activation energy (E_a), enthalpy of activation ($\Delta H^{\ddagger}$), free energy of activation ($\Delta G^{\ddagger}$) and entropy of activation ($\Delta S^{\ddagger}$) were calculated and were found to be 34.7, 32.2, 89.9 kJ mol^{-1} and -194 J mol^{-1} K^{-1} respectively. The lower energy of activation and high free energy of activation support the formation of highly solvated transition state. As the present reaction was undertaken in nonpolar solvents like DCM, greater solvation of the proposed nonpolar transition state compared to that of the reactants is expected. Furthermore, if the transition state is more extensively solvated than the reactants, $\Delta S^{\ddagger}$ assumes a larger negative value due to appreciable increase in ordering in the solvation shell.[42] These results support the formation of a nonpolar cyclic transition state in the rate-determining step.

5.3.4 Effect of surfactant

Lipophilic oxidants having long-chain quaternary ammonium counterion are known to form contact ion pairs in nonpolar solvents. As discussed in Chapter 1, these surfactants can form reverse micelles in nonpolar solvents, and mixed reverse micelles with various ionic and nonionic surfactants. The selectivity and mildness of these oxidants are proposed to be mainly because of the contact ion pair formation and formation of organized assemblies. To see the compatibility of CTAP in the formation of mixed aggregates with other surfactants and the effect of these aggregations on the reactivity and mechanism of CBZ oxidation, the effect of [surfactant] on the reaction rate was evaluated by using a cationic (CTAB), an anionic (SDS) and a non-ionic (TX-100) surfactant. With an increase in the concentration of surfactants, keeping the concentrations of all reactants constant, the rate of the reaction was found to increase in case of the ionic surfactants CTAB and SDS (Figure 5.8 and 5.9). The addition of TX-100 did not exhibit any significant change in the rate constant.

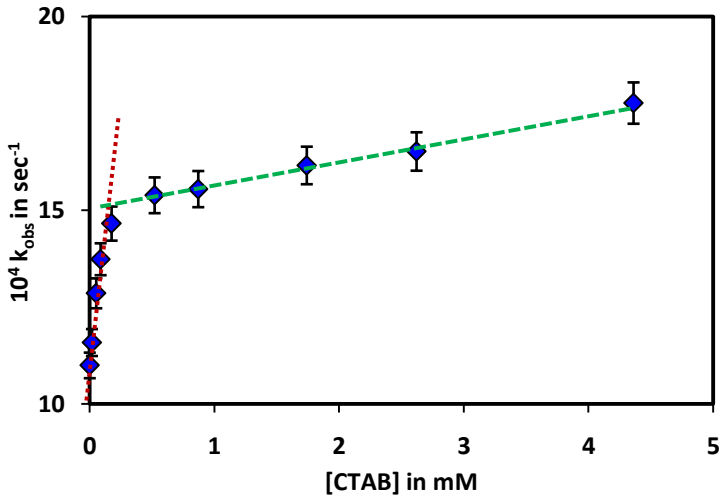


Figure 5.8 Effect of [CTAB] on the observed rate constants in the oxidation reaction of carbamazepine by CTAP at 298 K. (The dotted lines are drawn manually to indicate the variations).

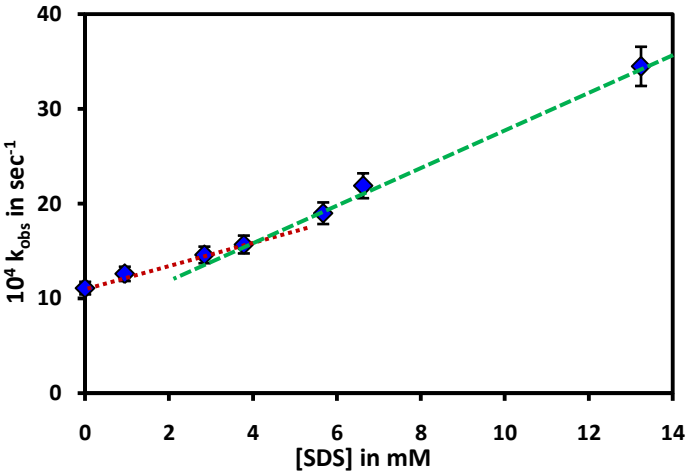
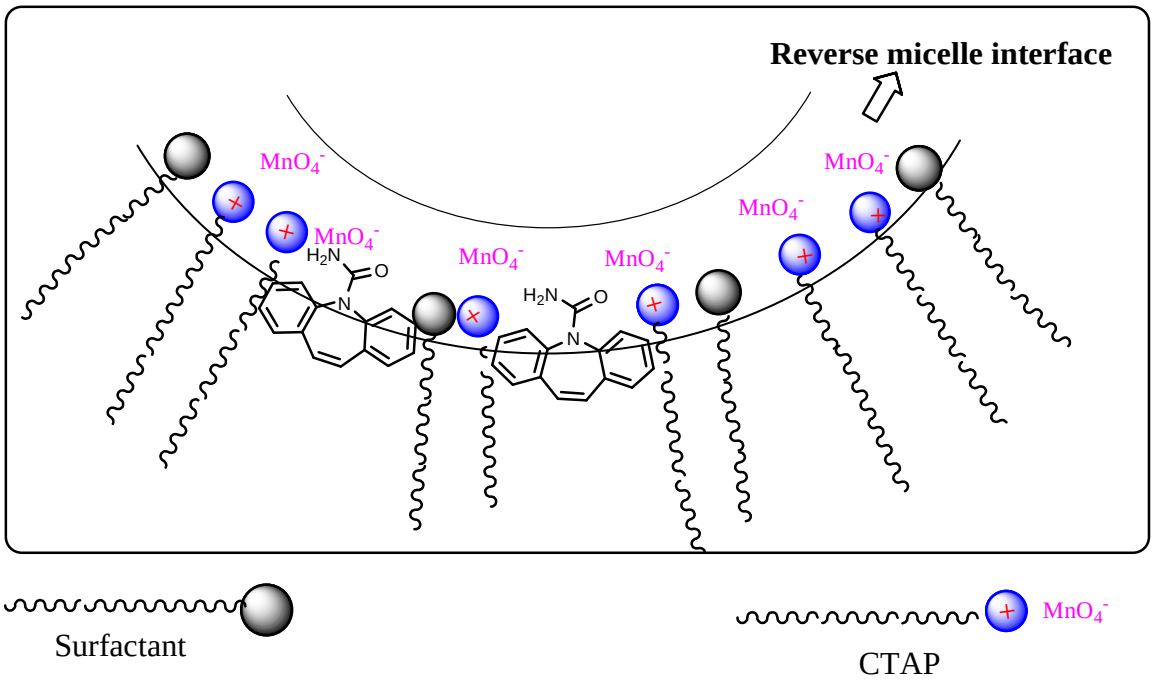


Figure 5.9 Effect of [SDS] on the observed rate constants in the oxidation reaction of carbamazepine by CTAP at 298 K. (The dotted lines are drawn manually to indicate the variations).

As shown in Figure 5.8 and 5.9, a bilinear increase in the rate constant was observed with an increase in the concentration of each of the surfactant with break points at around 0.2 and 4 mM for CTAB and SDS, respectively. With an increase in the concentration of CTAB from 0 to 0.2 mM, the rate constant increased sharply from 11 x 10^{-4} to 15 x $10^{-4}s^{-1}$. Beyond the concentration of 0.2 mM, it is found to increase slowly. In case of SDS, the increasing trend is opposite to that of the CTAB. Here, there is a slow increase in the rate constant initially at low concentration of SDS (up to 2.8 mM), beyond that a sharp increase is observed. The break point in the plots demonstrates the formation of mixed aggregates. The catalyzing effect of these surfactants can thus be explained through the greater partition of CBZ to the interfacial region, and hence the effective concentration of CBZ in the vicinity of MnO_4^- becomes higher resulting in an increase in rate. This phenomenon is represented schematically in Scheme 5.6. As compared to CTAB, in the presence of SDS, the rate constant is found to be higher in the higher concentration range. In the SDS-CTAP reverse micellar aggregate, cationic CTA^+ interacts with anionic dodecylsulphate. Owing to this interaction, the contactness between CTA^+ and MnO_4^- may decrease and the permanganate ion become free and thus is more available to the CBZ with a higher reactivity.



(**Scheme 5.6)**

5.4 CONCLUSION

CTAP oxidizes carbamazepine in DCM medium to produce a selective product 1*H*-dibenzo[*b, f*]azepine-4,5-dione. From the kinetic analysis, the reaction is found to be first order with respect to CTAP and fractional order with respect to CBZ. A suitable mechanism is proposed consisting of a first rate-determining *syn* addition of permanganate to the C=C double bond of CBZ to form Mn(V)-ester intermediate through a nonpolar cyclic transition state. The intermediate was further decomposed and hydrolyzed to the dicarbonyl product. The proposed mechanism is also supported from the effect of medium and effect of temperature on reaction rate. Ionic surfactants are found to catalyze the reaction due to the formation of mixed reverse micellar aggregates with CTAP.

5.5 REFERENCES

1. Bernus, I.; Dickinson, R. G.; Hooper, W. D.; Eadie, M. *J. Epilepsy Res.* **1996**, *24*, 163.
2. Leucht, S.; Mc-Grath, J. J.; White, P.; Kissling, W. *Cochrane Library Systematic Reviews* **2007**, *3*, 1.
3. Mattson, R. H. *Epilepsia* **2007**, *36*, 13.
4. Bailer, M.; Levy, R. H.; Perucca, E. *Ther. Drug Monit.* **1998**, *20*, 56.
5. Sternebring, B.; Liden, A.; Andersson, K.; Melander, A. *Eur. J. Clin. Pharmacol.* **1992**, *43*, 393.
6. Montgomery, S. A.; Schatzberg, A. F.; Guelfi, J. D.; Nemeroff, C.; Swann, A.; Zaiecka, J. *J Affect. Disord*. **2000**, *59*, S39.
7. Bertschy, G.; Bryois, C.; Bondolfi, G.; Velardi, A.; Burdy, P.; Dascal, D.; Martinet, C.; Baetting, D.; Baumann, P. *Pharmacol. Res.* **1997**, *35*, 451.
8. Crill, W. E. *Ann. Intern. Med.* **1973**, *79*, 844.
9. Gerson, W. T.; Fine, D. G.; Spielberg, S. P.; Sensenbrenner, L. L. *Blood*. **1983**, *61*, 889.
10. Horowitz, S.; Patwardhan, B.; Marcell, E. *Epilepsia* **1988**, *29*, 149.
11. Hart, R. G.; Easton, J. D. *Ann. Neurol*. **1982**, *11*, 309.
12. Leeder, J. S. *Epilepsia* **1998**, *39*, S8.
13. Kalapos, M. P. *Adverse Drug React. Toxicol. Rev.* **2002**, *21*, 123.
14. Romero, M. N.; Sendra, T. J.; Raboso, G. B. E.; Harto, C. A. *Eur. J. Dermatol.* **2002**, *12*, 503.
15. Riley, R. J.; Kitteringham, N. R.; Park, B. K. *J. Clin. Pharmacol*. **1989**, *28*, 482.
16. Shear, N. H.; Spielberg, S. P.; Cannon, M.; Miller, M. *J. Clin. Invest*. **1988**, *82*, 1826.
17. Park, B. K.; Coleman, J. W.; Kitteringham, N. R. *Biochem. Pharmacol.* **1987**, *36*, 581.
18. Ping, K.; Mingxiang, L.; Michael R.; Wester, J.; Steven, L.; Robin, E. P.; Maria, A. C. *Drug metab. Dispos.* **2008**, *36*, 490.
19. Kerr, B. M.; Thummel, K. E.; Wurden, C. J.; Klein, S. M.; Kroetz, D. L.; Gonzalez, F. J.; Levy, R. H. *Biochem. Pharmacol.* **1994**, *47*, 1969.
20. Valentine, C. R.; Valentine, J. L.; Seng, J.; Leakey, J.; Casciano, D. *Cell Biol. Toxicol.* **1996**, *12*, 155.
21. Hubin, T. J.; McCormick, J. M.; Collinson, S. R.; Buchalova, M.; Perkins, C. M.; Alcock, N. W.; Kahol, P. K.; Raghunathan, A.; Busch, D. H. *J. Am. Chem. Soc.* **2000**, *122*, 2512.
22. John, T.; Groves, J. L.; Marla, S. S. *J. Am. Chem. Soc.* **1997**, *119*, 6269.

23. Wietzerbin, K.; Muller, J. G.; Jameton, R. A.; Pratviel, G.; Bernadou, J.; Meunier, B.; Burrows, C. *J. Inorg. Chem.* **1999**, *38*, 4123.
24. Nam, W.; Kim, I.; Lim, M. H.; Choi, H. J.; Lee, J. S.; Jang, H. G. *Chem. Eur. J.* **2002**, *8*, 2067.
25. Mac Leod, T. C. O.; Barros, V. P.; Faria, A. L.; Schiavon, M. A.; Yoshida, I. V. P. Queiroz, M. E. C.; Assis, M. D. *J. Mol. Catal. A Chem.* **2007**, *273*, 259.
26. Williams, P. A.; Cosme, J.; Vinkovic, D. M.; Ward, A.; Angove, H. C.; Day, P. J.; Vonrhein, C.; Tickle, I. J.; Jhoti, H. *Science* **2004**, *305*, 683.
27. Ekroos, M.; Sjogren, T. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 13682.
28. Yano, J. K.; Wester, M. R.; Schoch, G. A.; Griffin, K. J.; Stout, C. D.; Johnson, E. F.; *J. Biol. Chem.* **2004**, *279*, 38091.
29. Dash, S.; Mishra, B. K. *Indian J. Chem.* **1997**, *36A*, 662.
30. Khan, A. A. P.; Asiri, A. M.; Azum, N.; Rub, M. A.; Khan, A.; Al-Youbi, A. O. *Ind. Eng. Chem. Res.* **2012**, *51*, 4819.
31. Hegde, R. N.; Shetti, N. P. ; Nandibewoor, S. T. *Ind. Eng. Chem. Res.* **2009**, *48*, 7025.
32. Dash, S.; Patel, S.; Mishra, B. K. *Tetrahedron* **2009**, *65*, 707.
33. Hu, L.; Martin, H. M.; Arce-Bulted, O.; Sugihara, M. N.; Keating, K. A.; Strathmann, T. *J. Environ. Sci. Technol.* **2009**, *43*, 509.
34. Rao, Y. F.; Chu, W.; Wang, Y. R. *Appl. Catal. A.* **2013**, *468*, 240.
35. Lee, D. G.; Brownridge, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 3033.
36. Freeman, F.; Kappos, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 6628.
37. Lee, D. G.; Brown, K. C. *J. Am. Chem. Soc.* **1982**, *104*, 5076.
38. Wiberg, K. B.; Geer, R. D. *J. Am. Chem. Soc.* **1966,** *88*, 5827.
39. Wiberg, K. B.; Saegebarth, K. A. *J. Am. Chem. Soc*. **1957**, *79*, 2822.
40. Lee, D. G.; Chen, T. *J. Am. Chem. Soc.* **1989**, *111*, 7534.
41. Rankin, K. N.; Liu, Q.; Hendry, J.; Yee, H.; Noureldin, N. A.; Lee, D. G. *Tetrahedron Lett.* **1998**, *39*, 1095.
42. Ruff, F.; Csizmadia, I. G. Organic reactions, Equillibria, kinetics and mechanism; Elsevier: New York, 1994.

Chapter 6

Oxidation of Norfloxacin by Cetyltrimethylammonium Permanganate

6.1 INTRODUCTION

Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid] is a representative member of fluoroquinolone family and widely used as a first choice of drug for the treatment of bacterial infections of the urinary, biliary, and respiratory tracts.[1,2] The antibacterial activity of fluoroquinolones arises due to the inhibition of growth of DNA gyrase (a critical enzyme to bacterial chromosome replication) of gram-positive and gram-negative bacteria through the formation of a ternary complex between the drug, the enzyme and bound DNA segment.[3-7] Carbonyl and carboxyl moiety of norfloxacin bind with DNA, break the DNA-gyrase complex and are responsible for the antibacterial activity. Fluorine (F) at 6^{th} position and piperazine substituent at 7^{th} position involve in binding interaction with enzyme, giving extra stabilization to the drug-enzyme-DNA ternary complex, and thus responsible for the increase in the effectiveness and potency of the antibacterial.[3-7] Norfloxacin is poorly metabolized in the body. 26-32% of the administered dose remained as such and excreted by renal excretion while only 5-8% of the drug is metabolized mainly to six active metabolites of lesser antimicrobial potency.[8] As studied in human and fungi, the drug is primarily metabolized in the liver by P450 enzymes via N-formylation/acetylation, oxidation and breakdown of the piperazine ring resulting in decreased antimicrobial activity.[9-12] Further, the bioavailability and thus the antibacterial activity of fluoroquinolones drastically decreased by the presence of multivalent metal containing compounds (such as magnesium or aluminium antacid, vitamins or minerals) at gastric pH and is attributed to the formation of metal-chelate complex and alternations in dissolutions.[13-14] In view of potential pharmaceutical importance of norfloxacin and presence of few literatures on the mechanism of oxidation of this drug[15-17] there is a need to understand the mode of interaction with the oxidizing agents and mechanism of oxidative metabolism of this drug in biomimetic medium.

Objectives: In view of the above, the present investigation is an attempt to utilize CTAP to look into the mechanism of the oxidative cleavage of norfloxacin and to investigate the following hypothesis.

- Will CTAP be used as mild, selective and biomimetic oxidants for the oxidative metabolism of norfloxacin?
- If so, what will be the reason of selectivity?
- What will be mechanism of this oxidative degradation? Are there any similarities with that of other biomimetic oxidations of norfloxacin in terms of mechanism?
- What will be the effect if any of different parameters such as reaction temperature, solvent polarity/hydrophobicity etc. on the overall rate and mechanism of the oxidative metabolism?

Kinetic method was used to study the mechanism. Basing on the kinetic data a rate expression and a plausible mechanism have been proposed. Effect of solvent on the reaction rate has been analyzed in media of varied polarities to suggest a suitable solvation model.

6.2 EXPERIMENTAL

6.2.1 Materials

Cetyltrimethylammonium permanganate (CTAP) was synthesized by following the reported procedure[18] as discussed in Chapter 5. Norfloxacin was purchased from Sigma-Aldrich, India and was used without further purification. Acetonitrile (Merck, India) was purified by standard method. Acetic acid (Merck, India) was used as source of hydrogen ions and was also used as such. D_2O (Sigma-Aldrich) used is of NMR grade with 99.8% D.

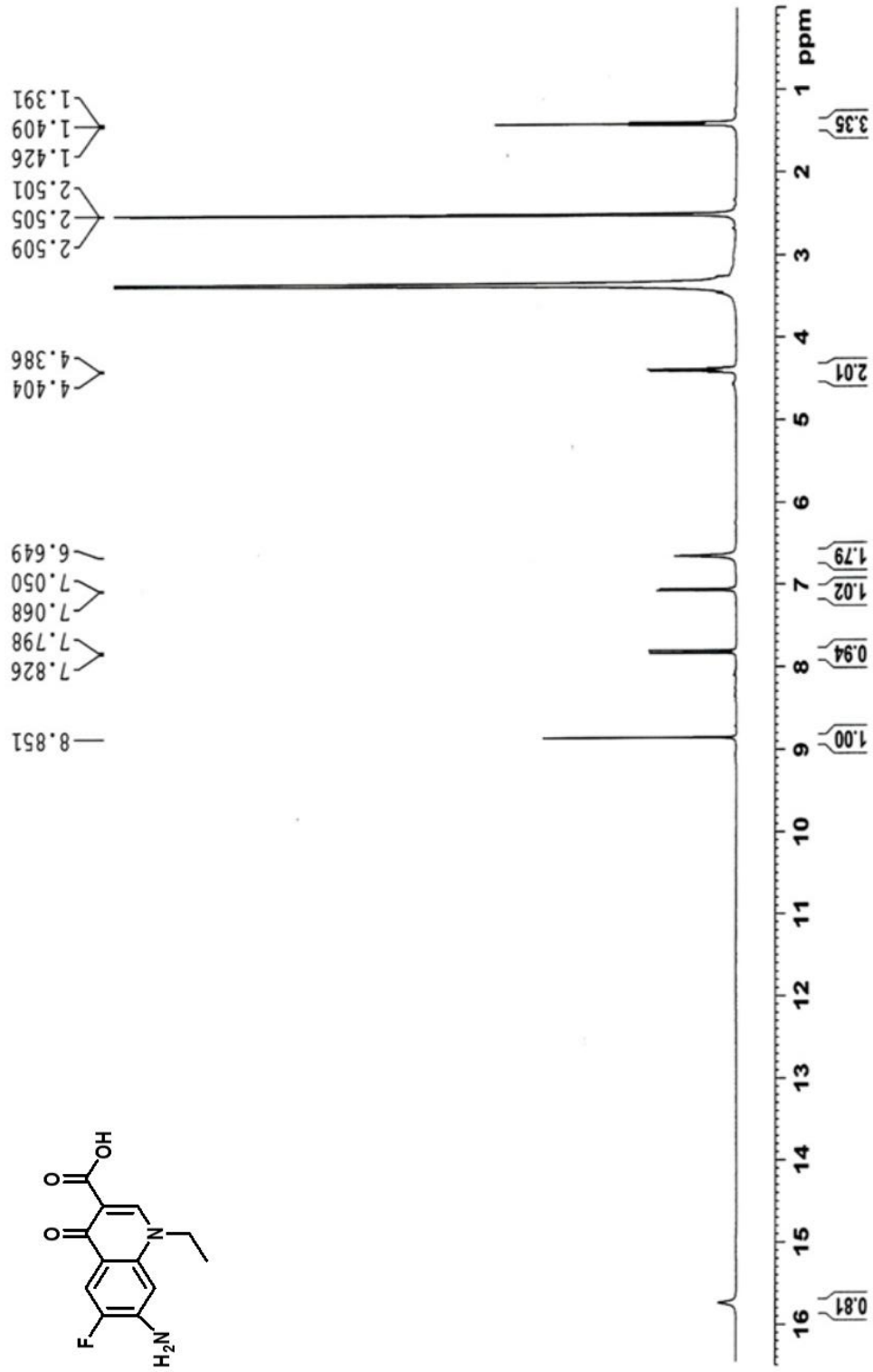


Figure 6.1 ^{1}H-NMR spectrum of 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid.

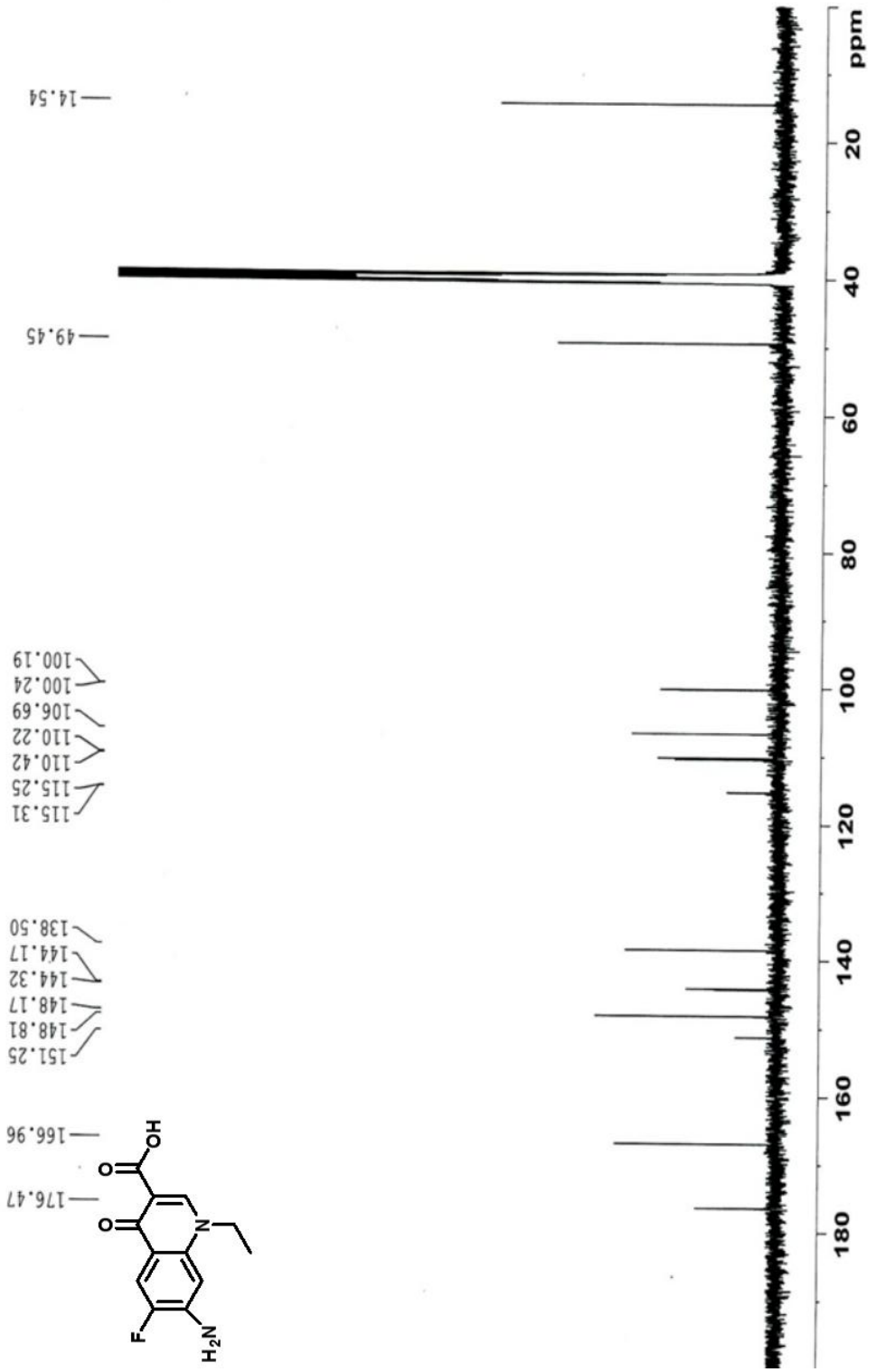


Figure 6.2 ^{13}C-NMR spectrum of 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid.

6.2.2 Kinetic measurements

The oxidation kinetics of norfloxacin by CTAP in acetonitrile-water solvent mixture in presence of acetic acid was investigated by monitoring the decrease in optical density (OD) of Mn(VII) at an analytical wavelength of 530 nm using Shimadzu UV-vis spectrophotometer (UV-1800) fitted with thermostatic cell holders. The temperature in the reaction cell was controlled by circulating water by using a Lauda thermostat within a temperature fluctuation of ±0.05 ^{o}C. All the kinetic runs were performed under pseudo-first-order conditions by keeping excess of the Norfloxacin (10 times or more) with respect to [CTAP]. The observed rate constants (k_{obs}), were obtained from the linear plots (R^2 = 0.99) of log [CTAP] against time for up to 75% completion of the reaction. The effect of the variation of [CTAP], [acid], and [norfloxacin] on the rate constant was investigated by varying the concentration of the desired constituent in the reaction mixture and the values reported are the average of at least triplicate runs and were reproducible within 6% error. The solvent isotope effect ($k(H_2O)/k(D_2O)$) was investigated using mixtures of H_2O-acetonitrile and D_2O-acetonitrile separately in presence of acetic acid at the mole fraction ratio of H_2O (or D_2O) : Acetonitrile: Acetic acid as 0.71:0.25:0.04.

6.2.3 Product analysis

The reaction mixture containing norfloxacin (64 mg, 1M) and CTAP (80 mg, 1M) in acetonitrile-water medium was stirred and refluxed for 15 minutes in the presence of 5% acetic acid. The reaction mixture turned brown, indicating the formation of reduced Mn(IV).[19] The completion of the reaction was monitored by TLC. The brown colored precipitate was separated from the reaction mixture by filtration and the filtrate was subjected to column chromatographic separation using chloroform and methanol as solvent. The pale yellow solid product thus obtained (Yield: 49 mg, 98%; M. Pt. 230°C) was characterized by NMR and mass spectral analysis. ^{1}H and ^{13}C NMR spectrum were recorded on a Bruker 400 MHz NMR

spectrometer. Mass data were obtained on a Perkin Elmer, Flexar SQ 300 MS Detector ESI Mass Spectrometer using acetonitrile as mobile phase at a flow rate of 10 ml/min. ^{1}H NMR (400 MHz, DMSO-d6): δ/ppm 15.75 (1H, S, -COOH), 8.85 (1H, S, C_2-H), 7.8 (1H, d, C_5-H), 7.1 (1H, d, C_8-H), 6.649 (2H, S, $–NH_2$ proton), 4.4 (2H, m, $–CH_2$), 1.426 (3H, t, $–CH_3$) (Figure 6.1); ^{13}C NMR (100 MHz, DMSO-d6): δ/ppm 176.47, 166.96, 151.25, 148.81, 144.32, 138.50, 115.31, 110.42, 106.69, 100.24, 49.45, 14.54 (Figure 6.2); MS (ESI, positive mode): m/z (relative intensity) 251 ($[M+H]^+$, 100). NMR and ESI-MS spectral analysis of the product agreed well with that of 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid. From TLC spot analysis, formaldehyde was found to be one of the degraded products. It was also confirmed by comparing the melting point of 2,4-dinitrophenylhydrazone derivative (M. Pt. 150 ^{o}C) with the literature value (M. Pt 156^{o}C).

6.2.4 Stoichiometry

The stoichiometry of the reaction was determined by performing the experiment at 298 K, under the condition of [CTAP] > [norfloxacin] at varying norfloxacin concentration keeping all other conditions constant. After 1 h, the concentration of remaining Mn(VII) was measured by monitoring the absorbance at 530 nm. A stoichiometry ratio of Norfloxacin/CTAP = 0.75, was observed. The stoichiometry of the reaction was also determined by limiting logarithmic method.[20] In this method, two different sets of experiments were done. In the first set of experiment, the norfloxacin concentration was varied (3.27×10^{-6} to 3.25×10^{-5} M) at a fixed CTAP concentration (0.9×10^{-3} M) and in the second set of experiment the CTAP concentration was varied (5.45×10^{-4} to 1.45×10^{-3}M) at a fixed norfloxacin concentration (3.27×10^{-6}M). The absorbances of Mn(VII) at 530 nm were recorded after 1 h of addition of reactants. The logarithms of the obtained absorbances were plotted against log [norfloxacin] and log [CTAP] in the first and the second set of experiments respectively. The slopes of the fitting line in both sets of experiments were calculated and found to be 0.433 and 0.574, respectively (Figure 6.3). Thus, the

molar reactivity of the reaction is 0.433/0.574, i.e. the reaction proceeds in the ratio of around 0.76:1 with respect to norfloxacin and CTAP.

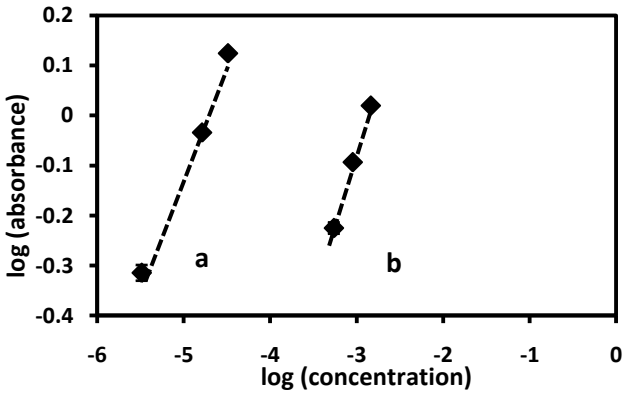


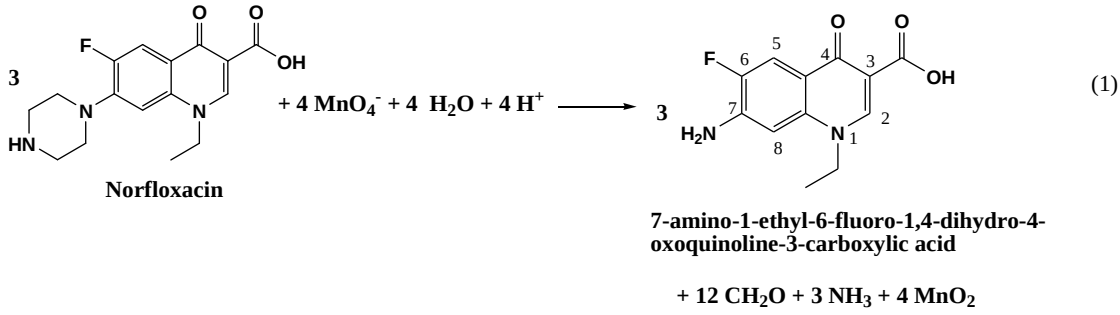
Figure 6.3 Limiting logarithmic plot for the calculation of the stoichiometric ratio between Norfloxacin and CTAP: (a) log absorbance vs. log [Norfloxacin] and (b) log absorbance vs. log [CTAP].

6.3 RESULTS AND DISCUSSION

The reaction mixture consisting of CTAP and norfloxacin in acetonitrile-water mixture in the presence of acetic acid became brown after 15 minutes under reflux condition. The main reaction products were identified as Mn(IV), 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, ammonia, and formaldehyde. The product, 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid was isolated with the help of column chromatographic separation technique and characterized by ESI-MS, ^{1}H NMR and ^{13}C NMR spectral analysis. Evolution of ammonia during the reaction was detected by its characteristic smell and litmus test. Presence of brown colored precipitate settled on the bottom of the flask after completion of the reaction indicated the formation of reduced Mn(IV). Formation of formaldehyde as one of the degraded product was confirmed by TLC spot test and comparing the melting point of the 2,4-dinitrophenylhydrazone derivative with that of the authentic sample.

Stoichiometry analysis revealed the reaction of 1 mol of CTAP with that of 0.75 mol of norfloxacin. The possibility of free radical mechanism may be ruled out

for the present reaction as (i) oxidation of norfloxacin, in an atmosphere of nitrogen, failed to induce polymerization of acrylonitrile[21] and (ii) the addition of acrylonitrile did not affect the rate of oxidation reaction.[20] Thus it may be proposed that, during the oxidation process, Mn(VII) is reduced to Mn(V), and finally converted to Mn(IV) by a disproportionation reaction in a sequential manner.[22] The stoichiometric equation is formulated as in Equation 1.



Norfloxacin

7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

(1)

In the oxidative transformation of norfloxacin using alkaline $KMnO_4$, Naik *et al.* have reported the insertion of OH radical at C2 of the quinolone ring to form 1-ethyl-6-fluoro-2-hydroxy-4-oxo-7-piperazin-1-yl-1,4-hydro-quinoline-3-carboxylic acid as the main reaction product.[17] Srivastava *et al.*, in the oxidative degradation of norfloxacin by the oxidant chloramine-T in aqueous micellar medium, reported the cleavage of C2-C3 bond to form 3-fluoro-4-piperazinoyl-6-N-ethylaminophenylglyoxalic acid as the main reaction product.[16] Similar oxidation product of norfloxacin have also been isolated by Nanda *et al.* using N-chlorosuccinimide as oxidant in acidic medium.[15] Though norfloxacin has many reaction sites, by using the present oxidizing system, it is converted to a single product. Similar to biological oxidant (P450), in the present oxidizing system, norfloxacin is attacked and metabolized selectively at the piperazinyl ring. This indicates the mildness, chemoselectivity and biomimetic characteristic of the oxidizing system toward the oxidation of norfloxacin.

For the mechanistic analysis of the oxidation reaction of norfloxacin by CTAP, the reaction kinetics was investigated using UV-vis spectroscopic technique.

Pseudo-first-order conditions were maintained by keeping excess of Norfloxacin with respect to [CTAP] unless otherwise stated. The rates of reactions were monitored by observing the rate of depletion of the absorbance peak at 530 nm (Figure 6.4). All reactions obeyed first-order kinetics. The observed rate constants (k_{obs}) were calculated from the linear plots of log [CTAP] versus time and presented in Table 6.1. Rates of the reaction at varying concentration of the oxidant, substrate and acetic acid were also calculated by multiplying [oxidant] with that of the corresponding k_{obs}.

Table 6.1 Effect of [Norfloxacin], [CTAP], and [Acetic acid] on the oxidation of norfloxacin by CTAP in acetonitrile-water mixture (mole fraction ratio 0.65:0.3) at 298K.

[CTAP] x 10^4 M	**[Norfloxacin] x 10^3 M**	**[Acetic acid] M**	**10^4 x k_{obs} s^{-1}**
0.1	1.8	1.27	19.60 ± 0.65
0.2	1.8	1.27	20.20 ± 0.68
0.4	1.8	1.27	19.23 ± 0.63
0.7	1.8	1.27	19.64±0.65
1.1	1.8	1.27	18.73 ± 0.6
1.8	1.8	1.27	19.00 ± 0.6
1.8	0.6	1.27	7.92 ± 0.34
1.8	3.7	1.27	39.62 ± 1.31
1.8	5.5	1.27	53.00 ± 2.19
1.8	7.3	1.27	58.46 ± 2.20
1.8	9.0	1.27	60.95 ± 2.35
1.8	10.2	1.27	65.70 ± 2.84
1.8	13.8	1.27	68.00 ± 3.34
1.8	1.8	0.16	5.50 ± 0.24

1.8	1.8	0.32	7.80 ± 0.31
1.8	1.8	0.63	13.50 ± 0.58
1.8	1.8	0.95	17.31± 0.75
1.8	1.8	1.58	19.54 ± 0.71
1.8	1.8	1.90	21.23± 0.70

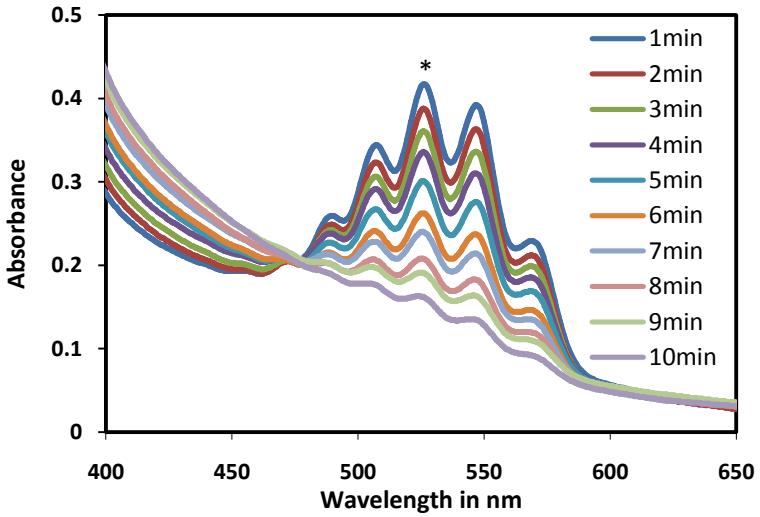


Figure 6.4 Successive scan of UV-vis absorption of the reaction mixture consisting of CTAP and norfloxacin in acetonitrile-water mixture in the presence of acetic acid at a time interval of 1 min. (* represents the peak taken for the calculation of k_{obs}).

6.3.1 Effect of reactant concentration

To observe the effect of CTAP concentration on the rate of the reaction, CTAP concentration was varied in the range of 1.1 x 10^{-5} to 5.45 x 10^{-4} mol dm^{-3}, at constant concentration of norfloxacin (1.8 x 10^{-3} mol dm^{-3}) and acetic acid (1.27 mol dm^{-3}). The linear plots of log (absorbance) versus time for different concentrations of CTAP indicated a first-order dependency with respect to CTAP. Further, the constant values of k_{obs} with change in [CTAP] also confirmed the reaction to be first-order with respect to CTAP (Table 6.1).

The individual reaction orders with respect to the norfloxacin and acetic acid were determined from the slopes of log (rate) versus log (concentration) plots constructed by varying the concentrations of respective reactants and were found to be 0.7 and 0.6 respectively. Further, the log (rate) values obtained at different conditions (Table 6.1) were subjected to multiple regression analysis, and were correlated with the parameters of the reaction condition, i.e. [CTAP], [norfloxacin] and [acetic acid] to examine the interdependency of reactant concentrations toward the rate of the reaction. The linear plot (R^2 = 0.99) of log (observed rate) versus log (predicted rate) (Figure 6.5) supported the regression model (equation 2). Using the coefficients obtained from equation 2, the rate expression can be obtained as in equation 3. The regression model confirmed the reaction to be first-order with respect to CTAP and fractional order with respect to norfloxacin and acetic acid.

$$\text{Log (rate)} = -0.82(\pm0.13) + 0.99(\pm0.03) \log [\text{CTAP}] + 0.73\ (\pm0.03) \log [\text{Norfloxacin}] + 0.59\ (\pm0.04) \log [\text{Acetic acid}] \quad (2)$$

R^2 = 0.99, F = 741, n = 21

$$\text{Rate} = 0.15\ [\text{CTAP}]^{0.99}\ [\text{Norfloxacin}]^{0.73}\ [\text{Acetic acid}]^{0.59} \quad (3)$$

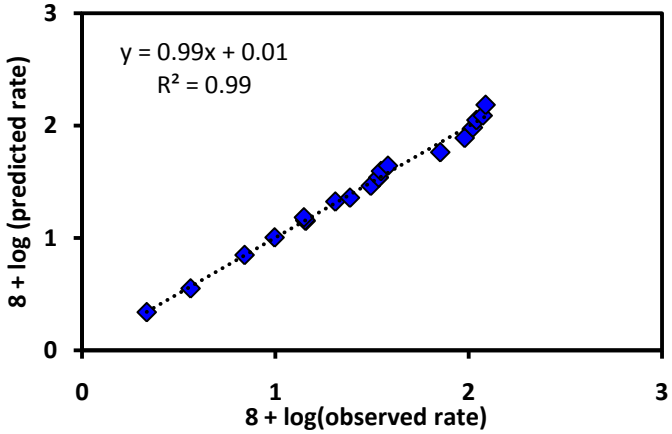


Figure 6.5 Plot of log (observed rate) versus log (predicted rate) for the oxidation reaction of norfloxacin with CTAP in acetonitrile-water mixture in the presence of acetic acid at 298 K.

The observed rate constant was found to increase with an increase in the [acetic acid] with almost no reaction in the absence of acid and tended toward a limiting value at higher acidities. It indicates the involvement of protonation of permanganate and existence of a rapid equilibrium with the protonated form in the oxidation process.[23] The protonation may be due to an exchange of CTA^+ with H^+ in the acetonitrile-H_2O-acetic acid medium. At higher acidities, protonation is almost complete leading to a limiting rate.

With an increase in concentrations of norfloxacin, the observed rate constant was found to increase asymptotically approaching its maximum value at higher concentrations (Figure 6.6A). Thus, a Michaelis-Menten type kinetics was proposed for the oxidation reaction(equation 4 and 5) i.e. the reaction involves the binding of oxidant and substrate to form a complex prior to the rate-determining step which subsequently decomposes to the products. Applying steady-state approximation to equation 4 and 5, the rate expression can be written as in equation 6.

$$\text{Norfloxacin} + \text{Oxidant} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} [\text{Complex}] \tag{4}$$

$$[\text{Complex}] \xrightarrow[\text{rds}]{k_2} \text{Product} \tag{5}$$

$$\frac{1}{k_{obs}} = \frac{1}{k_2\, K[\text{Norfloxacin}]} + \frac{1}{k_2} \tag{6}$$

From the Lineweaver-Burk double reciprocal plot (Figure 6.6 B) k_2, K ($=k_{+1}/k_{-1}$) and K_m were calculated to be 10.5 x 10^{-3} s^{-1}, 147.3 $dm^3\,M^{-1}$, and 6.8 x 10^{-3} M dm^{-3} respectively. The Michaelis-Menten constant K_m (steady-state dissociation constant of the oxidant-substrate complex) is an inverse measure of the substrate's affinity for the catalyst (enzyme). Smaller the K_m, higher is the affinity of the catalyst for the substrate. To measure the substrate affinity for the oxidant, K_m values were measured at different acid concentration at constant oxidant concentration. With the variation in

acetic acid concentration, no significant change in K_m values was observed, delineating no effect of acetic acid concentration either in the complex formation or in the decomposition of the complex.

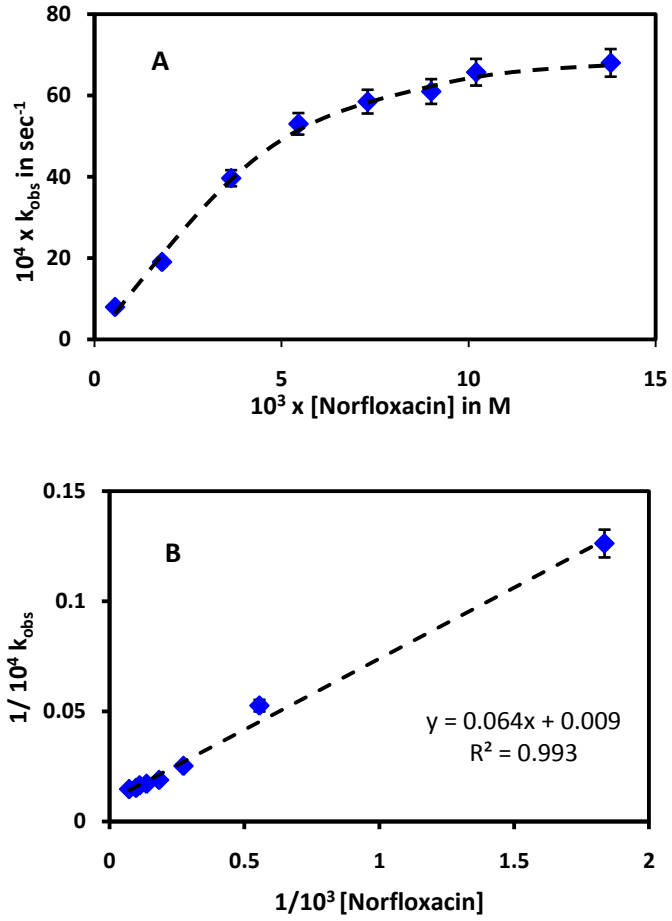
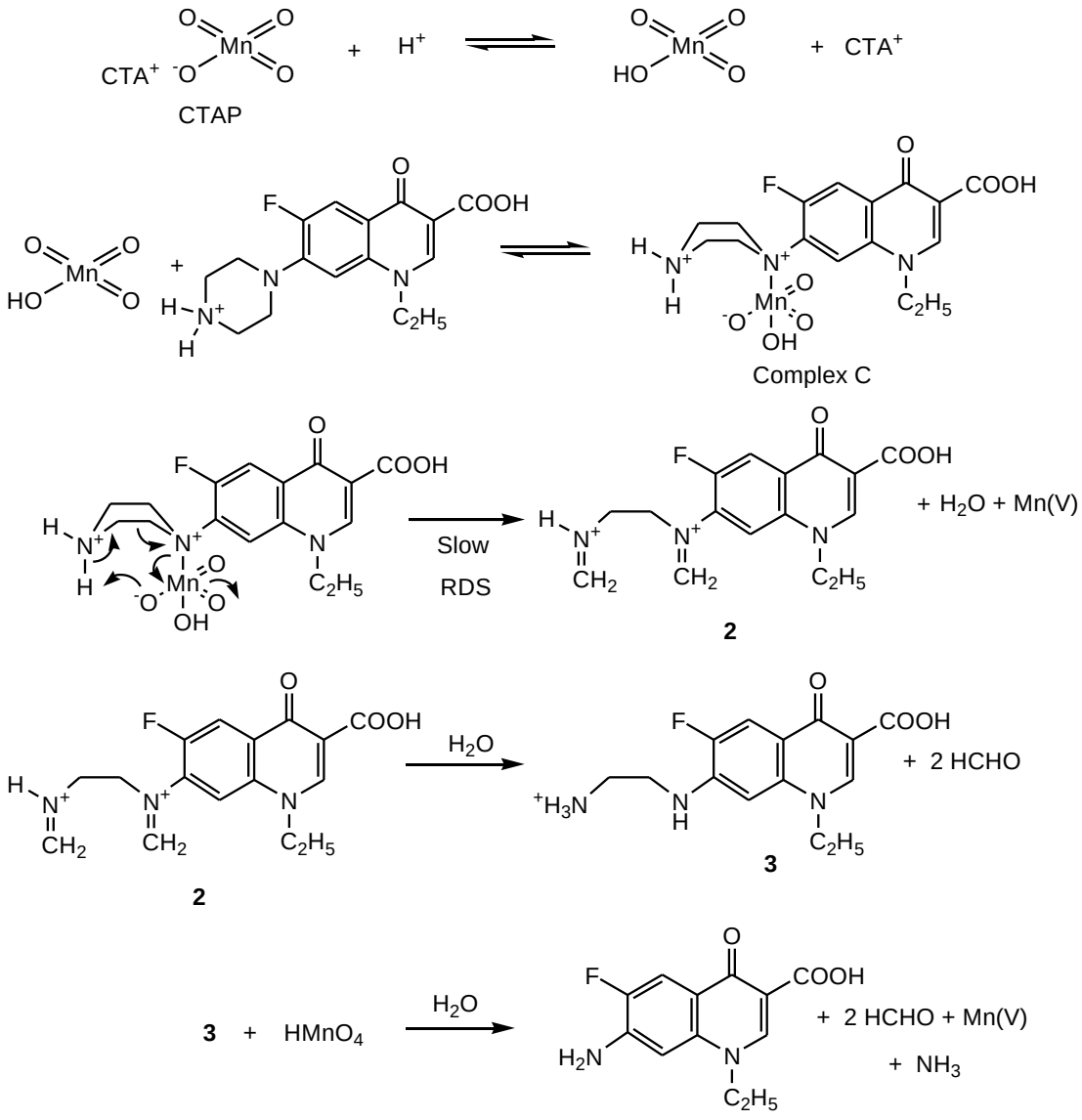


Figure 6.6 (A) Plot of 10^4 x k_{obs} versus 10^3 x [norfloxacin] and (**B**) Plot of $1/(10^4$ x $k_{obs})$ versus $1/(10^3$ x [norfloxacin]) in the oxidation reaction of norfloxacin by CTAP in acetic acid-acetonitrile-water medium (0.05:0.65:0.3) at 298 K.

In view of these experimental findings, a plausible mechanism can be proposed as in Scheme 6.1**,** wherein the first step involves the protonation of CTAP followed by formation of a complex with norfloxacin in a second equilibrium step. In a slow rate-determining step, the complex then decomposes to form Mn(V) and the intermediate iminium cation (**2**). The intermediate undergoes hydrolysis in a further fast step to form formaldehyde and another intermediate (**3**). **3** subsequently reacts with the another mole of Mn(VII) in a similar manner followed by the hydrolysis of

the corresponding iminium cation to form the final product 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid along with ammonia and formaldehyde.

The oxidant-substrate complex is formed through the interaction between electronegative nitrogen (N1) of the piperazine ring and electropositive manganese of the permanganate. Though N4 nitrogen of piperazine ring has higher electron density than N1 of norfloxacin, it is not expected to take part in the complex formation since it exists in protonated form in acidic pH.[24]



(Scheme 6.1)

6.3.2 Effect of solvent polarity

Investigation of effects of solvent on reactivity always plays a crucial role in determining the nature of the transition state and the mechanism of the reaction. In the light of the transition-state theory, the change in the rate of a reaction with change in the nature of the solvents or the polarity of the solvents is mainly due to difference in solvation pattern and relative stabilization of the starting material and the corresponding activated complex by the solvents.[25,26] Increase in solvent polarity either increases or decreases the reaction rate, depending on whether the activated complex is more or less dipolar than the initial state. Further, a change in solvent polarity has a negligible effect on the rates of reactions that involve little or no change in the charge density on going from reactants to the activated complex.[25,26]

In the present investigation, the oxidation reaction was carried out in solutions containing varying proportions of acetonitrile (dipolar aprotic solvent) and water (polar protic solvent) in the presence of a fixed amount of acetic acid (5%). The observed rate constant was found to show significant solvent sensitivity (Table 6.2). To analyze the solvent induced change in reactivity, k_{obs} were correlated with different solvent parameters such as ε (dielectric constant),[27] π* (solvent polarity),[28] β (hydrogen bond acceptor basicity),[29] α (hydrogen bond donor acidity), [29] A (anion solvating power of the solvent),[30] B (cation solvating power of the solvent),[30] and A+ B (solvent polarity).[30] The polarity parameters for the solvent mixtures have been estimated approximately from the polarity parameters of the pure solvents.

Table 6.2 Observed rate constants for the oxidation of norfloxacin by CTAP in the acetonitrile- water solvent mixture in the presence of acetic acid at 298 K. [CTAP]= 1.8 x 10^{-4} M, [Norfloxacin] = 1.8 x 10^{-3} M, [Acetic acid] = 1.27 M.

Mole fraction (CH_3CN)	Mole fraction (water)	Mole fraction (acetic acid)	ε	A	B	α	β	10^4x k_{obs} (s^{-1})	8+ln k_{obs} (s^{-1})
0.80	0.14	0.06	41.50	0.49	0.84	0.35	0.27	125.50 ± 5.44	3.62
0.72	0.23	0.06	44.96	0.54	0.85	0.43	0.26	45.42 ± 1.80	2.61
0.65	0.30	0.05	48.04	0.59	0.86	0.49	0.25	19.00 ±0.60	1.73
0.62	0.33	0.05	49.45	0.61	0.87	0.52	0.25	14.32 ±0.55	1.45
0.59	0.36	0.05	50.79	0.63	0.87	0.55	0.25	9.75 ± 0.32	1.07
0.56	0.39	0.05	52.05	0.65	0.88	0.58	0.24	7.60 ± 0.33	0.82
0.43	0.52	0.04	57.52	0.72	0.90	0.70	0.23	6.50 ± 0.28	0.66
0.33	0.63	0.04	61.87	0.79	0.92	0.80	0.22	5.90± 0.35	0.56
0.25	0.71	0.04	65.41	0.84	0.93	0.88	0.21	5.72 ± 0.34	0.53

With an increase in dielectric constant of the medium, k_{obs} was found to decrease suggesting a relatively less polar transition state with smaller charge density than the reactants (Figure 6.7).[33] On increasing the dielectric constant of the medium from 41 to 52, a sharp decrease in rate constant is observed. On the other hand, in the region of higher dielectric constant (>52) the rate constant is virtually independent of the composition. This kind of non-ideal decrease demonstrates preferential solvation of reactants and transition state by either of the solvents through specific and non-specific solute-solvent interaction such as hydrogen bonding.[31] The change of trend in the reactivity with change in solvent composition may also be due to a change in the mechanism. However, the validity of isokinetic relationship for the whole series of solvent composition in the present case with an isokinetic temperature of 236 ± 17 K confirms that the bilinear decreasing trend with change in solvent composition is not due to a change in the reaction mechanism.[32]

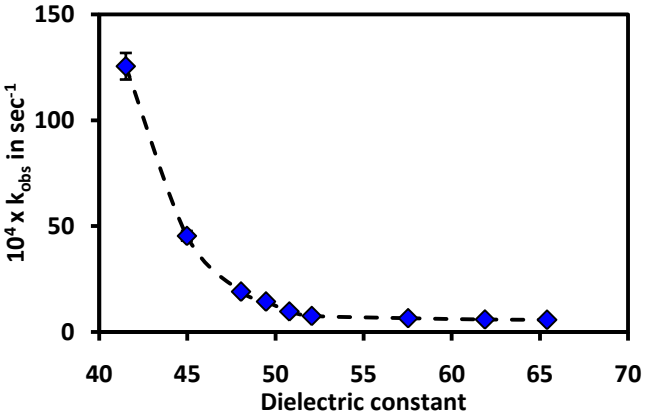
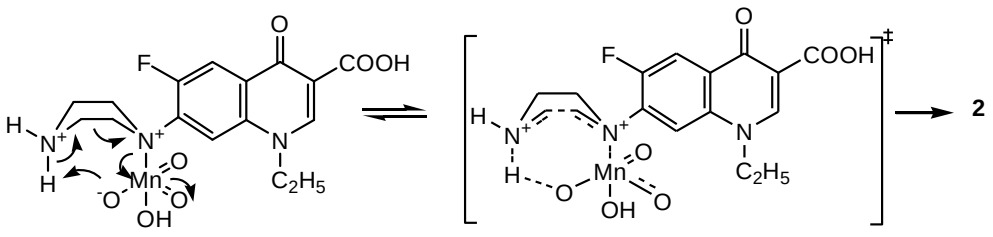


Figure 6.7 Plot of 10^4 x k_{obs} versus dielectric constant of the medium (ε) for the oxidation of norfloxacin by CTAP in the presence of acetic acid (1.27 M) in acetonitrile-water solvent mixtures.

The decrease in rate constant with an increase in ion solvating power (A+B) of the medium depicts the involvement of ions in the rate-determining step. Further, with the increase in both A and B, similar decreasing trend is observed, which may propose the presence of both cation and anion in the complex C and the transition state in the rate-determining step.

Based on all these experimental observations, it may be proposed that in the rate determining step the complex C decomposes to the products through a less polar transition state as represented in Scheme 6.2. The transition state is expected to be less polar than the complex C due to delocalization of the negative charge.



(Scheme 6.2)

Figure 6.8 demonstrates a bilinear decrease in ln k with change in solvent composition. The rate constants are found to be higher in the higher mole fraction of

acetonitrile, decreased sharply with further increase in water content in the mixture and became constant at higher mole fraction of water (mole fraction > 0.4). This kind of bilinear decrease may be ascribed to the differential solvation of the complex **C** and the transition state (**4**) through specific and nonspecific solute solvent interaction.

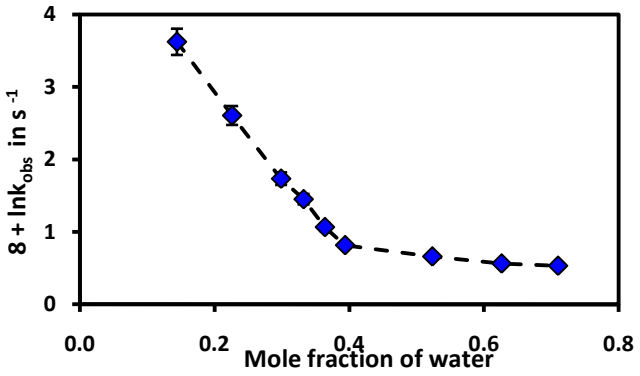


Figure 6.8 Plot of 8 + ln k_{obs} versus mole fraction of water in the oxidation of norfloxacin by CTAP in presence of acetic acid (1.27 M) in acetonitrile-water solvent mixtures.

Higher rate of reaction in the larger acetonitrile domain can be explained by greater solvation of less polar transition state (TS) by less polar acetonitrile compared to that of water. The positive charges in the transition state are localized on both the nitrogens while the negative charge is delocalized through the piperazine ring and manganate moiety. Accordingly, the transition state **4** might behave more as a cation than an anion. Being dipolar aprotic and due to the presence of nonbonding electrons, acetonitrile acts as a good cation solvator (B= 0.86),[26,30] and it can strongly solvate the TS through ion-dipole and dispersive interactions leading to greater stabilization of the TS. In contrast, being a poor anion solvator (A = 0.37),[26,30] it will not be able to solvate the complex C so extensively compared to that of the less polar TS. Consequently, stability of the complex will be less in acetonitrile domain, which will increase the rate of decomposition. On the other hand, being polar protic and having greater anion solvating power (A =1),[38] water solvates the complex C more extensively than the TS. Thus, stability of complex C will be more in water rich domain which will decrease the rate of the reaction.

For reactions involving ions and dipolar molecules, ln k is related to the dielectric constant of the medium (ε) as per equation 7, where k_0 refers to rate constant in the medium of unit dielectric constant, rand $r^{\neq}$ refer to radii of the reacting species and the activated complex respectively.[26]

$$lnk = lnk_0 + \frac{NZ^2e^2}{2\varepsilon RT}\left(\frac{1}{r} - \frac{1}{r^{\neq}}\right) \quad (7)$$

As per the above equation, ln k is linearly dependent on 1/ε. The slope of the line depends on the radii (r and $r^{\neq}$) of the reactants and the transition states and accordingly the rate of the reaction will either increase or decrease with the increase in solvent polarity. If the activated complex is highly solvated than the reactants, $r^{\neq}$ will be greater than r and the slope increases with the increase in solvation. On the other hand, if the reactant is highly solvated than the value of r will increase leading to a decrease in the magnitude of the slope. The bilinearity in the plot of ln k versus 1/ε (Figure 6.9) confirmed the validity of the equation for the present investigation in both the acetonitrile rich domain (ln k = 577(1/ε) -10. 26: R^2 = 0.999) and water rich domain (ln k = 75(1/ε) -0.62: R^2 = 0.983). Greater sensitivity of the rate constant with higher positive slope in the acetonitrile rich domain supports the presence of highly solvated activated complex than the reactants.[26] In water rich domain (mole fraction of water > 0.39), however, the reactant is preferentially solvated by water through ion-dipole interaction and hydrogen bonding.

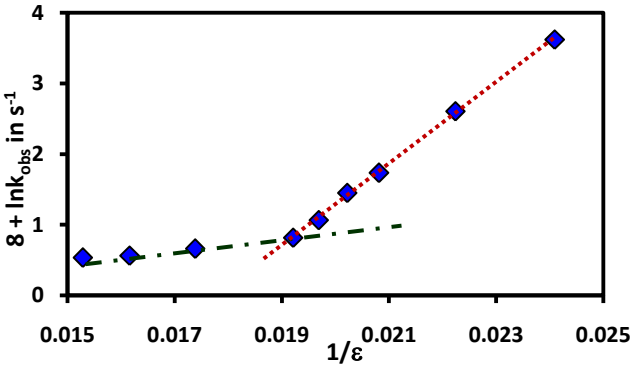


Figure 6.9 Plot of 8 + ln k versus. 1/ε in the oxidation of norfloxacin by CTAP in the presence of acetic acid (1.27 M) in acetonitrile-water binary solvent mixtures.

With the increase in β (HBA ability) value and decrease in α (HBD ability) value of the medium, ln k was found to increase (Figure 6.10). These facts illustrate the involvement of hydrogen bonding between the solute (reactant and/or transition state) and solvent molecules, which leads to preferential solvation. Water, being a polar protic solvent can act as a strong hydrogen bond donor as well as hydrogen bond acceptor.[33-35] It can form hydrogen bonds with N-H protons of the piperazine moiety as HBA as well as with manganate anion as HBD solvent. On the other hand, acetonitrile being a non HBD and a weak HBA solvent, it may forms only weak hydrogen bond with N-H proton of the piperazine moiety. As a result of which strong intramolecular hydrogen bonding between manganate anion and NH^+ of piperazine moiety may prevail. As a result in the oxidant-substrate complex (C), two types of hydrogen bonding causing difference in rate are expected (i) strong intermolecular hydrogen bonding between manganate anion and water in water rich domain and (ii) strong intramolecular hydrogen bonding between manganate anion and NH^+ of piperazine moiety in acetonitrile rich domain (Scheme 6.3).

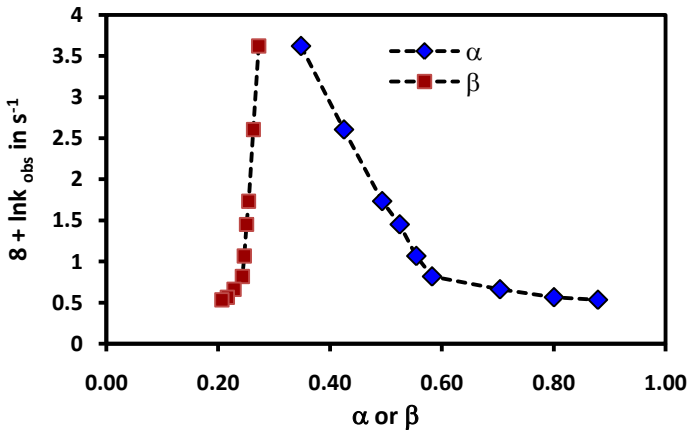
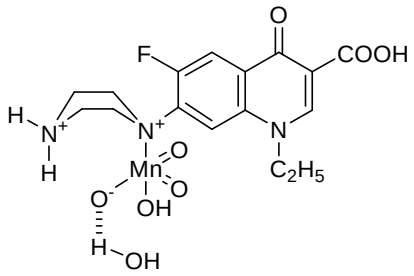
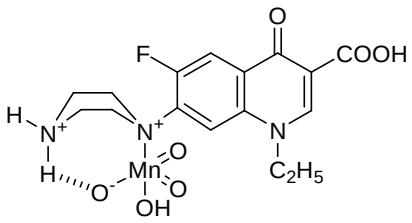


Figure 6.10 Plot of 8 + ln k versus α and β of the solvent mixture in the oxidation of norfloxacin by CTAP in the presence of acetic acid (1.27 M) in acetonitrile-water medium at 298K.



Strong intermolecular hydrogen bonding in water rich domain



Strong intramolecular hydrogen bonding in acetonitrile rich domain

(Scheme 6.3)

As shown in Scheme 6.3, stronger intramolecular hydrogen bonding in the oxidant-substrate complex leads to higher rate of hydrogen abstraction from piperazine moiety and thus the rate of the reaction is higher. Due to strong HBD ability water can preferentially solvate the oxidant-substrate complex and stabilize the complex through intermolecular hydrogen bonding, which leads to a slower rate of decomposition of the complex.

6.3.3 Effect of temperature

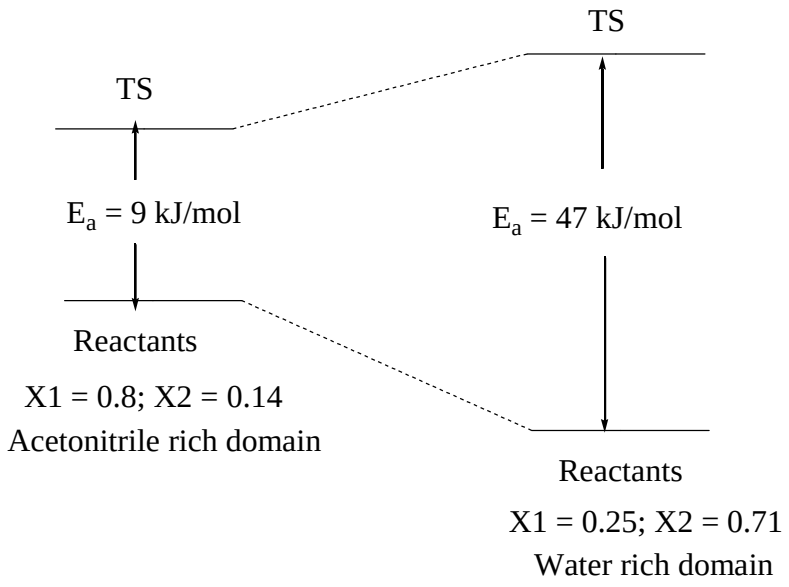
The proposed mechanism was further supported by the effect of temperature on the rate of the reaction. With the increase in temperature from 293 to 313 K, the rate constant was found to increase linearly (Table 6.3). The thermodynamic parameters were calculated using Arrhenius and Eyring equation (Table 6.3). The lower energy of activation and high free energy of activation support the formation of highly solvated transition state.[36] The high negative entropy of activation is also indicative of an extensively solvated and highly ordered activated complex than the reactants. It also supports the existence of a cyclic or a forced transition state during abstraction of N-H proton by permanganate anion and subsequent breaking of C-C bond of the complex C in the rate determining step (Scheme 6.2).[37]

Table 6.3 Rate constants at three different temperatures and activation parameters for the oxidation of norfloxacin by CTAP in acetonitrile-water solvent mixture in the presence of acetic acid. [CTAP]= 1.8 x 10^{-4} M, [Norfloxacin] = 1.8 x 10^{-3} M, [Acetic acid] = 1.27 M

Mole fraction (CH_3CN) X1	Mole fraction (water) X2	10^4 k_{obs} (s^{-1})			E_a kJ mol^{-1}	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta G^{\neq}$ kJ mol^{-1}	$\Delta S^{\neq}$ J mol^{-1} K^{-1}
		298K	303K	313K				
0.80	0.14	125.5	140	151	9.1	6.5	85.1	-259
0.72	0.23	45.42	52.2	59	13	10.5	87.54	-254
0.65	0.30	19	22.6	26.8	17.2	14.67	89.76	-248
0.62	0.33	14.32	17.27	21.11	19.5	16.96	90.49	-242
0.59	0.36	9.75	10.75	19.96	38.59	36.04	91.28	-182
0.56	0.39	7.6	8.44	16.54	41.89	39.35	91.87	-173
0.43	0.52	6.5	7.53	14.75	43.77	41.22	92.2	-168
0.33	0.63	5.9	6.4	13.6	45.31	42.77	92.6	-164
0.25	0.71	5.72	6.14	13.6	47.13	44.6	92.6	-158

To further support the above proposal, activation parameters were calculated at different solvent composition (Table 6.3). The energy of activation (E_a) was found to be smaller in the acetonitrile rich domain and was also found to increase systematically with the increase in the water content of the solvent mixture. These facts further supported the following; (i) Stronger is the intramolecular hydrogen bonding lesser is the E_a for hydrogen abstraction and higher is the rate of reaction (ii) stronger is the intermolecular hydrogen bonding with water, higher is the stability of oxidant-substrate complex and slower is the rate of decomposition. Further high polar nature of water may destabilize the nonpolar transition state compared to that of acetonitrile. This phenomenon is presented graphically in Scheme 6.4. The lower the temperature, the stronger is the preferential solvation of the probe molecule by the solvents.[38] An increase in temperature results in increased kinetic energy of molecules, and thereby the interaction between molecules connected with preferential

solvation becomes weaker. The hydrogen bonding between the reactants and solvents also weakens resulting in an increase in the rate of reaction.



(Scheme 6.4)

With an increase in the mole fraction of water from 0.14 to 0.71, the entropy of activation ($\Delta S^{\neq}$) was found to increase from -259 to -158 J K^{-1} mol^{-1}. The values of $\Delta S^{\neq}$ are generally influenced by the degree of solvation and solvent polarity. If the transition state is more extensively solvated than the reactants, $\Delta S^{\neq}$ assumes a larger negative value due to an appreciable increase in ordering in the solvation shell.[39] Thus, the higher negative value of $\Delta S^{\neq}$ in acetonitrile rich domain as compared to water rich domain may be attributed to the greater solvation of the nonpolar transition state in the less polar solvent.

6.3.4 Determination of solvent kinetic isotope effect

Isotopic labeling on solvents influences the rate of the reaction and gives useful information regarding the reaction mechanism. To obtain the solvent kinetic isotope effect ($k(H_2O)/k(D_2O)$), the reaction was carried out in the mixture of H_2O-acetonitrile and D_2O-acetonitrile separately. The solvent kinetic isotope effect, k (H_2O)/k (D_2O), was found to be 1.18(± 0.05). The magnitude of the isotope effect, in

this case, is similar to that of a secondary isotope effect and may be due to the following factors.[35]

(i) A secondary isotope effect is observed if the easily exchangeable protons of the reactants are replaced by deuterium, but the deuterium-containing bonds are not broken during the rate-determining step of the reaction.

(ii) Secondly, secondary isotope effect is observed if the solvent (water in the present case) performs a nucleophilic attack in the rate-determining step.

(iii) With isotopic substitution, the physical characteristics of the solvent also change. Consequently, the solute-solvent interaction will be somewhat different, altering the strength of H-bonds or nucleophilicity of the reactants resulting in secondary kinetic isotope effect.

In the preset case, the observed secondary solvent kinetic isotope effect can be explained through the difference in solute-solvent interaction due to isotopic substitution as the hydrogen bond strength is higher in D_2O compared to that of water.[40] Thus, it is expected that, the oxidant substrate complex forms stronger hydrogen bonds with D_2O compared to water leading to increase in the energy of activation. Consequently, the solvent kinetic isotope effect is greater than one though considerably smaller.

6.4 CONCLUSION

Norfloxacin was metabolized by CTAP in aqueous acetonitrile medium in the presence of acetic acid to produce 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, formaldehyde and ammonia through selective attack at the piperazine ring. From the kinetic analysis, the reaction was found to be first order with respect to CTAP and fractional order with respect to norfloxacin and acetic acid. A suitable reaction mechanism was proposed, the first step of which is a very fast formation of association complex between oxidant and substrate, followed by a rate determining dissociation of the complex proceeding via a less polar cyclic

transition state. The proposed mechanism is supported by the effect of solvent, solvent kinetic isotope effect and from the effect of temperature on the reaction rate. The outcome of solvent effect illustrates the presence of a differential contribution from dipolar aprotic and polar protic solvents toward the reaction through specific and nonspecific solute-solvent interaction. The study could through some light on the fate of this drug in the presence of the oxidant like Mn(VII) in biological systems.

6. 5 REFERENCES

1. CoRdoba-Dı Az, M.; Co Rdoba-Borrego, M.; Co Rdoba-Dı Az, D. *J. Pharm. Biom. Anal.* **1998**, *18*, 865.
2. Bennet, P. N.; Brown, M. J. *Clin. Pharmacol.* **2005**, IX, 132.
3. Turiel, E.; Esteban, A. M.; Tadeo, J. L. *J. Chromat. A* **2007**, *97*, 1172.
4. Hooper, D. C. *Drugs* **1999**, 58, 6.
5. Palu, G.; Valisena, S.; Ciarrocchi, G.; Gatto, B.; Palumbo, M. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9671.
6. Sicci, C.; Andreolli, M.; Cecchetti, V.; Fravolini, A.; Gatto, B.; Palumbo, M. *Bioorg. Med. Chem.***1998**, *6*, 1555.
7. Sharma, P. C.; Jain, A.; Jain, S. *Acta. Poloniae. Pharma. Drug Res.* **2009**, *66*, 587.
8. Hooper, D. C.; Wolfson, J. S. *Antimicrob. Agents Chemother.* **1985**, *28*, 716.
9. Sharma, P.C.; Saneja, A.; Jain, S. *Int. J. Chem. Sci.* **2008**, *6*, 1702.
10. Pauliukonis, L. T.; Musson, D. G.; Bayne, W. F. *J. Pharm. Sci.* **1984**, *73*, 99.
11. Parshikov, I. A.; Heinze, T. M.; Moody, J. D.; Freeman, J. P.; Williams, A. J.; Sutherland, J. B. *Appl. Microbiol. Biotechnol.* **2001**, *56*, 474.
12. Abraham, D. J. *Medicinal Chemistry Drug Discovery*, John Wiley and Sons, Hoboken, New Jersey 2003 p. 582- 587.
13. Campbell, N. R.; Kara, M.; Hasinoff, B. B.; Haddara, W. M.; McKay D. W. *Br J. Clin. Pharmacol.* **1992**, *33*, 115.
14. Alkaysi, H. N.; Abdel-Hay, M. H.; Salem, M. S.; Gharaibeh, A. M.; Nawas, T. E. *Int. J. Pharm.***1992**, *87*, 73.
15. Nanda, N.; Kumar, P.; Malini, *Int. J. Pharm. Sci. Rev. Res.* **2013**, *23*, 388.
16. Shrivastava, A.; Singh, A. K.; Sachdev, N.; Shrivastava, D. R.; Katre, Y.; Singh, S. P.; Singh, M.; Mejuto, J. C. *J. Mol. Catal. A: Chem.* **2012**, *1*, 361.
17. Naik, P. N.; Chimatadar, S. A.; Nandibewoor, S.T. *Ind. Eng. Chem. Res.* **2009**, *48*, 2548.
18. Mishra, B. K.; Dash, S. *Indian J. Chem*. **2001**, *40A*, 159.

19. Tabassum, S.; Sabir, S.; Sulaiman, O.; Rafatullah, M.; Khan, I.; Hashim, R. *J. Disper. Sci. Tech.* **2011**, *32*, 217.
20. Hegde, R. N.; Shetti, N. P.; Nandibewoor, S. T. *Ind. Engg. Chem. Res.* **2009**, *48*, 7025.
21. Khan, A. A. P.; Asiri, A. M.; Azum, N.; Rub, M. A.; Khan, A.; Al-Youbi, A. O. *Ind. Eng. Chem. Res.* **2012**, 51, 4819.
22. Mohnot, K.; Sharma, P. K.; Banerji, K. K. *J. Org. Chem.* **1996**, *61*, 1310.
23. Byadagi, K. S.; Hosahalli, R. V.; Nandibewoor, S. T.; Chimatadar, S.A. *Ind. Eng. Chem. Res.* **2011**, *50*, 10962.
24. Musa, K. A. K.; Eriksson, L. A. *J. Phys. Chem. A*, **2009**, *113*, 10803.
25. Reichart, C. *Pure Appl. Chem* **1982**, *54*, 1867.
26. Ruff, F.; Csizmadia, I. G. *Organic reactions, Equillibria, kinetics and mechanism*, Elsevier, New York, 1994.
27. Riddick, J. A.; Bunger, W. B.; Organic Solvents; Techniques of Chemistry, vol. II, Wiley Interscience, New York, 1970.
28. Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.***1983**, *48*, 2877.
29. Kamlet, M. J.; Taft, R.W. *J. Am. Chem. Soc.* **1976**, *98*, 377.
30. Swain, C. G.; Swain, M. S.; Powel, A. L.; Alunni, S. *J. Am. Chem. Soc.***1983**, *105*, 502.
31. Patel, S.; Gorai, S.; Malik, P.K. *J. Photochem. Photobiol. A* **2011**, *219*, 76.
32. (a) Leffler, J. E. *J. Org. Chem.***1955**, *20*, 1202 (b) Exner, O. *Coll. Czech. Chem. Commun.* **1964**, *29*, 1094 (c) Mansoor, S. S.; Shafi, S.S. *J. Mol. Liq.* **2010**, *155*, 85.
33. Hojo, M. *Pure Appl. Chem*. **2008**, *80*, 1539.
34. Hojo, M.; Ueda, T.; Ueno, E.; Hamasaki, T.; Nakano, T. *Bull. Chem. Soc. Jpn.* **2010**, *83*, 401.
35. Hojo, M.; Uji-yie, Y.; Tsubota, S.; Tamura, M.; Yamamoto, M.; Okamura, K.; Isshiki, K. *J. Mol. Liq.* **2014**, *194*, 68.
36. Asiri, A. M.; Khan, A. A. P.; Khan, A. *J. Mol. Liq.* **2015**, *203*, 1. (b) Khan, A. A. P.; Mohd, A.; Bano, S.; Siddiqi, K. S. *Ind. Eng. Chem. Res.* **2011**, *50*, 9883.
37. Braisted, A.C.; Schultz, P.G. *J. Am. Chem. Soc.* **1990**, *112*, 7430.
38. (a) Martins, C. T.; Lima, M. S.; El Seoud, O. A. *J. Org. Chem.* **2006**, *71*, 9068. (b) Martins, C. T.; Lima, M. S.; Bastos, E. L.; El Seoud, O. A. *Eur. J. Org. Chem.* **2008**, 1165.
39. Khan, A. A. P.; Mohd, A.; Bano, S.; Siddiqi, K. S. *Ind. Eng. Chem. Res.* **2011**, *50*, 9883.
40. Scheiner, S.; Cuma, M. *J. Am. Chem. Soc.***1996**, *118*, 1511.

Chapter 7

Summary and Future Scope

7.1 SUMMARY

Oxidative transformations by lipopathic Cr(VI) and Mn(VII) containing quaternary ammonium ions are widely explored in synthetic organic chemistry and also a topic of current interest for the versatility as well as selectivity in oxidation of organic substrates in organic solvents. With appropriate alkyl groups in the quaternary ammonium groups, the salts assemble in both aqueous and nonaqueous media to form organized assemblies mimicking bioaggregates like protein, lipids, and nucleic acids. In this context the present thesis is an attempt to establish two lipopathic oxidants, CTADC and CTAP (containing long chain amphiphilic cetyltrimethylammonium ion as counter ions for dichromate and permanganate respectively) and to answer the following questions.

(i) Can these oxidants be used as mild, selective oxidants in the oxidation of multifunctional organic substrates?

(ii) Can these oxidants be used as biomimetic oxidants for the oxidation of biologically important organic substrates?

(iii) If so, can these be used as metal based non-enzymatic chemical models to study the mechanism of oxidative metabolism and to synthesize selective metabolites in sufficient amounts?

For the purpose, the oxidative metabolism of some established drugs (such as acetaminophen, epinephrine, isoniazid, carbamazepine and norfloxacin having multifunctional groups) have been investigated in nonaqueous medium. A brief summary of the findings are presented as follows.

- Formation of single, chemoselective and biomimetic products in the oxidation of above substrates demonstrates the mildness and selectivity of these oxidizing systems.

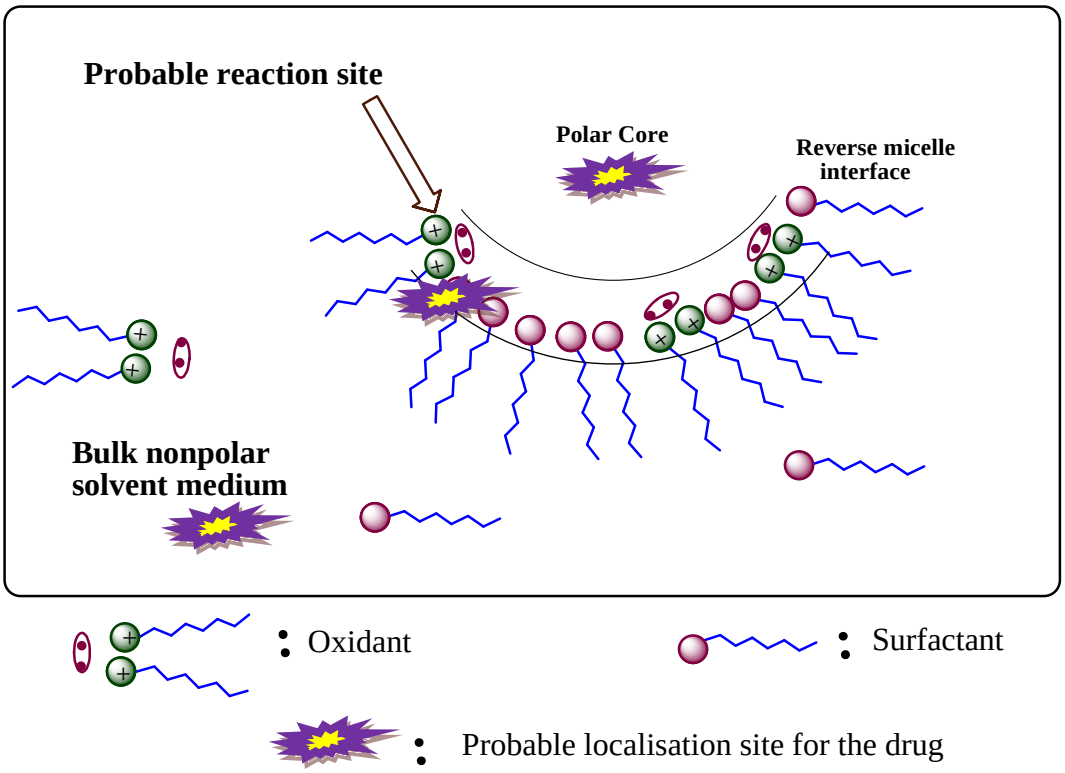
- These lipopathic oxidants containing long chain CTA counterions are found to be more advantageous than other classical and non-classical lipopathic oxidants of Cr(VI) and Mn(VII).
- The uniqueness in terms of reactivity, selectivity and difference with that of other classical and non-classical oxidants is attributed to the presence of CTA^+ which tunes the reactivity of the anionic oxidizing counterpart through tight ion pair formation in nonpolar medium.
- Kinetic analysis of the above oxidative conversions reveals similar mechanistic path for all reactions. Fractional order dependencies with respect to the drugs demonstrates the initiation of the reaction through the reversible binding between the oxidant and the substrate at selective site to form a complex which then decomposes to the product through subsequent reaction steps (Scheme 7.1).

Oxidant + Substrate $\rightleftharpoons$ **Oxidant-Substrate Complex** **Step 1**

Oxidant-Substrate Complex $\longrightarrow$ **Products** **Step 2**

(Scheme 7.1)

- Similar to that of enzyme catalysed reaction, these oxidants provide suitable residing/binding sites for proper orientation of the substrates as well as active site/catalytic site for reaction (Scheme 7.2). The oxidative conversion takes place predominantly at the interfacial region of the reverse micellar aggregates. Nature of solvents and the polarity of the solvents have been found to play significant roles in controlling the rate of the reaction either through controlling the aggregation process or by differential solvation of reactants and transition states through specific and non-specific solute-solvent interaction.



(Scheme 7.2)

- These oxidants may serve as a non-enzymatic, chemical oxidation model primarily to synthesize the selective metabolites of drug candidates in the course of drug discovery and development.
- Elucidation of probable mechanistic pathway of drug oxidation using these oxidants in presence of artificial organised assemblies may serve as a reasonable model to enlighten the complex biological reactions occurring at the lipid–solution interface in the living system and give valuable information for finding more suitable drug candidate to fight with oxidative stress and to combat drug resistance.

7.2 FUTURE SCOPE

In view of the success of the strategy to utilize long chain amphiphilic CTA ion as onium counter ion for the synthesis of lipopathic Cr(VI) and Mn(VII) oxidants for mild, selective and biomimetic oxidations, it can be extended to synthesize novel lipopathic Fe(III), Ru(VII), Mo(VI) and W(VI) oxidants for possible application in selective oxidative functional group transformations.

Due to the insolubility or poor solubility in aqueous medium these oxidants can be utilized as heterogeneous oxidizing system for treatment of contaminated stream or possible degradation and removal of xenobiotics.

Kinetic and spectrophotometric determination of drug in pharmaceutical formulations has been the subject of interest to many investigators due to their sensitivity, selectivity, simplicity and accuracy. In these methods the drug is oxidized using the metal ion oxidant and thus the amount of drug has been estimated. But in most of the methods high concentration of strong acid, high temperature and longer reaction time is required for the process. However, CTAP and CTADC oxidize many oxidizable functional groups in milder conditions as compared to other conventional oxidants. Further, due to the suitable reactivity of these oxidants, the kinetics of the reaction can be studied properly. Owing to the selectivity, sensitivity and accuracy of kinetic methods and due to the mildness of the oxidizing method, the oxidation kinetics of drug by these lipopathic oxidants can be a better alternative to the available methods for the determination of drug in pharmaceutical formulations.

In the development of new pharmaceuticals, one crucial task is to elucidate the metabolic fate of the drug candidate that need to be studied at early stages. Cell-based metabolism studies of active compounds using enzymes liver microsomes, hepatocytes or micro-fungi present several advantages but the difficulty of isolation from the biological medium and small quantities of the metabolites are severe drawbacks. Thus many non-enzymatic chemical models have been proposed as

biomimetic alternative approach to the cell-based metabolism study of bioactive compounds. In this regard, these oxidants may serve as a biomimetic, non-enzymatic, chemical oxidation model primarily to synthesize selective metabolites of drug candidates in the course of drug discovery and development. Further, elucidation of probable mechanistic pathway of drug oxidation using these oxidants in presence of artificial organized assemblies can serve as a reasonable model to enlighten the complex biological reactions occurring at the lipid–solution interfaces in the living system.

LIST OF PUBLICATIONS

1. **Sarita Garnayak and Sabita Patel,** Oxidative cleavage of acetaminophen by cetyltrimethyl ammonium dichromate: A mechanistic study. *Ind. Engg. Chem. Res*. **2013,** *52***,** 13645.
2. **Sarita Garnayak and Sabita Patel,** Oxidation of epinephrine by cetyltrimethylammonium dichromate: Mechanism of formation of adrenochrome. *Ind. Engg. Chem. Res*. **2014,** *53,* 12249**.**
3. **Sarita Garnayak and Sabita Patel,** Oxidation of carbamazepine by lipopathic oxidant, cetyltrimethylammonium permanganate: A mechanistic study. *Int. J. Chem. Kinet.* **2015**, *47*, 429.
4. **Sarita Garnayak and Sabita Patel,** Oxidative degradation of norfloxacin by a lipophilic oxidant, cetyltrimethylammonium permanganate in water-acetonitrile medium: A kinetic and mechanistic study. *J. Mol. Liq.* **2015,** *209,* 327.
5. **Sarita Garnayak and Sabita Patel,** Oxidation of antitubercular drug isoniazid by lipopathic oxidant, cetyltrimethylammonium dichromate: A mechanistic study. *Int. J. Chem. Kinet.* **2016**, *48*, 32.

Bio-Data
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Degree	Board/University	Year	Division	Subjects
10th	BSE, Odisha	1999	1st	Eng., Math, Science, Sans., MIL
+2 Science	CHSE, Odisha	2001	2nd	Phy., Chem,. Biol, Math.
+3 Science (Hons)	Sambalpur University	2005	1st	Chem (H), Phy, Math, Eng. MIL
M.Sc.	Sambalpur University	2007	1st	Org (E), Inorg, Phy
M.Phil	Sambalpur University	2008	1st	Organic Chem.

Awards:

- **Professor R. K. Nanda Award (2013)** for best paper presentation in the 27th Annual conference of "**Orissa Chemical Society".**
- **Best Poster presentation award** in the National seminar on "Advanced in Chemistry and their Biological and Industrial relevant" held in NIT-Rourkela, Jan. 10-11th **2014**.
- **Best Poster Presentation award** in 16th National Symposium in Chemistry (CRSI) Organized by IIT- Bombay, Feb. 6-9th **2014**.

Research experience:

April 2010 – Continuing	Doctoral Research on "Oxidation of some biologically important organic substrates by lipopathic Cr(VI) and Mn(VII): Kinetics and mechanistic study." **Research area:** Physical-Organic Chemistry. **Supervisor:** Dr. Sabita Patel, Department of Chemistry, National Institute of Technology Rourkela, India.
22nd Oct. 2008 – 23rd Feb. 2010	Project fellow in "Golden Triangle project." **Research area:** Standardization of ayurvedic formulation. **Supervisor:** Dr. U.V. Mallavadhani, Department of Natural products, CSIR lab, IMMT, Bhubaneswar.
December 2007 – December 2008	M. Phil in Organic Chemistry. **Research area:** Synthesis of spiroheterocycles containing thiobarbituric acid moiety. **Supervisor:** Prof. R.K. Behera, School of Chemistry, Sambalpur University, Odisha, India.

Conference attended:

- Oral presentation in RDCST, held at the National Institute Of Technology, Rourkela, Odisha (15th-16th March 2014)
- Poster presentation in National Conference in "Current Trend in Surface Science and Technology" held at Sambalpur University, School of Chemistry, Jyotivihar, Burla dated 28th Feb. 2014)
- Poster presentation in 16th National symposium in Chemistry (NSC-16) & 8th CRSI-RSC Symposium in Chemistry held at Indian Institute Technology Bombay (IIT-Bombay), (6-9 Feb. 2014)
- Poster presentation in National Conference in "Advanced in Chemistry and their Biological and Industrial Research" held at NIT-Rourkela, 10-11th Jan 2014.

- Oral presentation in 27th Annual Conference of Orissa Chemical Society held at the Modern Engineering and Management Study, Balasore, Odisha (14th-15th Dec. 2013)
- Poster presentation in 14th National symposium in Chemistry (NSC-14) & 6th CRSI-RSC Symposium in Chemistry held at National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram (2-5 Feb. 2012)
- Oral presentation in Silver Jubilee Annual conference of Orissa Chemical Society held at Sambalpur University (24-26th Dec, 2011)
- Seminar on "Role of Scientific Community in Pollution Prevention" held at National Institute Of Technology, Rourkela (2nd December 2011)
- National Seminar on Recent Trends in Chemical Sciences (RETICS) held at Sambalpur University, Orissa (28-29 March, 2009)
- Oral presentation in 22nd Annual Conference of Orissa Chemical Society & National Seminar on Materials Chemistry and Catalysis held at North Orissa University, Baripada (27-28 2008)
- National Seminar on Recent Trends in Chemical Sciences (RETICS) held at Sambalpur University, Orissa (23-25 March, 2008)
- National Seminar on Recent Trends in Chemical Sciences (RETICS) held at Sambalpur University, Orissa (23-25 March, 2007)
- National workshop on "Radiochemistry and Applications of Radioisotopes" held at Sambalpur University (17-23 Aug. 2006)

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1. **Sarita Garnayak and Sabita Patel,** Oxidative cleavage of acetaminophen by cetyltrimethyl ammonium dichromate: A mechanistic study. *Ind. Engg. Chem. Res*. **2013,** *52***,** 13645.
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3. **Anita Pati, Poulomi Majumdar, Sarita Garnayak, Ajay K. Behera, Rajani K. Behera**, Regiospecific MIRC reaction of 1,3-diphenylthiobarbituric acid and dimedone: Formation of spiro vs condensed heterocycles. *Indian J. Chem.B,* **2014**, *53B,* 384.
4. **Sarita Garnayak and Sabita Patel,** Oxidation of carbamazepine by lipopathic oxidant, cetyltrimethylammonium permanganate: A mechanistic study. *Int. J. Chem. Kinet.* **2015**, *47*, 429.
5. **Sarita Garnayak and Sabita Patel,** Oxidative degradation of norfloxacin by a lipophilic oxidant, cetyltrimethylammonium permanganate in water-acetonitrile medium: A kinetic and mechanistic study. *J. Mol. Liq.* **2015,** *209,* 327.
6. **Sarita Garnayak and Sabita Patel,** Oxidation of antitubercular drug isoniazid by lipopathic oxidant, cetyltrimethylammonium dichromate: A mechanistic study. *Int. J. Chem. Kinet.* **2016**, *48,* 32.